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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

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This Supplement to Volume 1 of *Neurobehavioral Toxicology* contains the Proceedings of the Workshop on "Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function," which was held April 1–4, 1979, at the Hilton Palacio del Rio Hotel in San Antonio, Texas. This meeting was organized by the Southwest Foundation for Research and Education with the direction of Dr. Irving Geller; and was sponsored by the United States Environmental Protection Agency, Office of Toxic Substances, under Contract No. EPA 68-01-4870. These Proceedings serve as the Final Report No. EPA 560/11-79-010. The opinions expressed in these articles, however, are those of the authors and are not necessarily endorsed nor shared by the Environmental Protection Agency.

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Supplement to *NEUROBEHAVIORAL TOXICOLOGY*

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Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function

Preface

The subject of this Workshop and the desired output is clearly stated in the title. It was held "to obtain a scientific assessment for regulatory decision-making of the currently available methodologies necessary to determine the toxic threat to human health and the environment posed by all chemicals in commerce." The Office of Toxic Substances of the Environmental Protection Agency is required in its enabling act, the Toxic Substances Control Act (TSCA), to carry out such determinations in the area of behavioral disorders, among others. This meeting was therefore initiated with the inspiration of Dr. Joseph Seifter.

When I became Project Officer for this Workshop, I soon realized how fortunate the Office of Toxic Substances was to have Irving Geller as the guiding hand to organize the meeting. It is indeed true to say that the meeting could not have succeeded without his outstanding knowledge of the behavioral field, and his untiring efforts to pull all the loose ends together. With the invaluable assistance of Dr. William Stebbins, he achieved a program which included the best international thinking and research in behavioral toxicology, and gave the EPA its best chance of succeeding in its prescribed responsibilities.

The various opinions I have heard indicate that this Workshop did succeed in delineating areas of agreement. To this extent, EPA can be confident that meaningful tests are available for measuring sensory and functional impairment, and beyond this, changes in the more complex areas of discriminant and learning processes and reproductive behavior. It has, however, clearly been confirmed that the full breadth of behavioral toxicology with its integration of complex interactions and compensatory mechanisms may never be fully circumscribable. In any case, since EPA must take into account the economic realities, it could not prescribe every one of a completely predictive set of tests since there would be so many, even if such a set could be developed.

The answer to achieving adequate guidelines for regulatory consistency may lie in some approaches which merit further exploration. The quantitative activities of various reference compounds in a selection of well-characterized tests treated with multivariate statistical methods may give profiles which delineate different types of neurobehavioral toxicity, and which may be useful in determining the qualitative toxicities of unknowns. Each such profile might then suggest the need for more extensive testing in certain critical areas of behavior. Then, as suggested by Weiss and Laties in the last paper, EPA by consultation with a scientific advisory board could decide whether the sponsor's proposed set of tests properly covers the likely areas of concern in light of the latest understanding of the discipline.

Finally, I must express my thanks for the valuable contributions of the session chairmen and all of the speakers who, of course, were the raison d'être of the meeting. And for this report, thanks again to Irv Geller, Bill Stebbins and Matt Wayner for the efficient editing process which has led so expeditiously to this publication. Only one contribution could not be included in this publication and the final material was received by the Publisher on August 13, 1979.

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Opening Remarks: TSCA Requirements for Testing Chemicals for Behavioral Effects and Neurotoxicity

NORBERT P. PAGE

Environmental Protection Agency

ON behalf of the Environmental Protection Agency (EPA) I join with Mr. Goland in welcoming you to this important workshop on Behavioral and Neurotoxicologic Test Methods. I particularly want to thank Dr. Irving Geller, Dr. William Stebbins, and Dr. David Gould for developing such a promising program. The actual need for this workshop was identified over two years ago, Dr. Joseph Seifter of the EPA and Dr. Geller of the Southwest Foundation for Research and Education providing the initiative to organize and enter the workshop into the EPA's program. The need for a successful workshop is even greater today than was realized then.

We have with us many national and international experts in the behavioral sciences and other areas of toxicology, and I look forward to an enlightening discussion of testing methods in behavioral and neurotoxicology. To provide some groundwork for this discussion and at the same time keep introductory remarks brief, my comments will be restricted primarily to three subjects: (1) the regulatory framework within which the workshop results might be utilized; (2) the EPA's responsibilities for testing under the Toxic Substances Control Act (TSCA) and its approach to implementing standards; and (3) the specific objectives or issues for consideration by this workshop.

The overall objective for the human behavioral workshop described in the announcement follows: "The workshop is to obtain a scientific assessment for regulatory decision-making of the currently available methodologies necessary to determine the toxic threat to human health and the environment posed by all chemicals in commerce." I would like to stress one part of that objective—the need for scientific assessment of currently available methodologies. Under TSCA, EPA has the authority and the responsibility to ensure manufacturers provide data on which the assessment of unreasonable risk can be made. Congress singled out several key health effects of concern, one of which was behavioral disorders.

CHEMICALS AS BEHAVIORAL AND NEUROTOXICITY DETERMINANTS

How big a role do chemicals play in the human behavioral disorder problem? Some scientists say their role is minimal. Others claim chemicals play a very major role which generally has gone unrecognized. To me it seems likely that behavioral disorders are the result of a complex array of many factors including genetics, nutritional aspects, our socioeconomic factors, diseases, and of course, chemicals. It is certain that some chemicals play a major role in the etiology of neurological or behavioral disorders. Several come to mind immediately: the role of heavy metals, such as lead and mercury, a number of the pesticides, including many organophosphates, and some of the chlorinated chemicals such as kepone. An important group that has been incriminated is the chlorinated solvents. I am sure as we move through this workshop we will hear of many other chemicals which have been shown to produce behavioral or neurotoxic effects.

METHODS OF ASSESSMENT

How can we assess for the behavioral or neurotoxicology potential of chemicals? One very obvious method is the association of conditions observed in humans with exposure to specific chemicals. Like all epidemiological studies, this approach is expensive to conduct. Moreover, it is difficult to sort through subjective complaints where exposures may be to several chemicals, and where many modifying factors interact. Indeed, this is not an easy task. Some papers to be presented in this workshop will deal with this aspect of human observation and surveillance. The other main category of assessment methods is the whole animal tests of various types. These range from general toxicity studies where behavior or neurological effects may be observed as a part of a routine health examination to the more sophisticated and specific or tailored tests which are designed to assess behavioral and neuromotor function in a more consistent manner.

REGULATORY FRAMEWORK FOR TSCA, SECTIONS 4 AND 5

The regulatory framework of TSCA is unique for Federal legislation in that it provides specific authority in Sections 4 and 5 to require manufacturers or processors to provide data to EPA for assessment purposes. Section 4 pertains to existing chemicals or categories of chemicals; whereas Section 5 pertains to new chemicals which are to be manufactured. Under Section 4, EPA issues chemical test rules that define a chemical or categories of chemicals which must be tested, the effect to be tested for, and the standards

by which the testing will be performed. Under Section 5, the Agency has no specific testing authority; however, a premanufacturing notification must be submitted to EPA 90 days prior to manufacture. In that premanufacturing notification, the manufacturer must provide data on which the Agency can make an assessment of risk to human health and the environment.

The approach we are using with Section 4 is to develop and place into the Code of Federal Regulations generic standards for various health effects. These standards will then be referenced at a later time when chemical test rules are proposed. At the time of proposing a chemical test rule, specific modifications to the generic standards can be made so as to customize the standards to the chemicals or category of chemicals to be tested. Moreover, the standards must be reviewed annually and revised as appropriate to assure their currency with scientific development.

A number of health effects testing standards have been developed and will shortly be proposed in the *Federal Register*. Four of these should be published by the end of April. These are testing for oncogenicity, nononcogenic chronic effects, all chronic effects and good laboratory practices. A number of other health standards should be proposed in the early summer. These are for acute toxicity including lethality, eye irritation, dermal sensitization and dermal irritation, subchronic toxicity testing, teratogenicity, reproductive effects, and mutagenicity. This latter group of standards will be proposed basically as they appear in the guidelines proposed last August for use in registration of pesticides under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

We have not made a decision on behavioral or neurotoxicity standards. The reason for this is simple. We are uncertain as to which test systems are well enough validated and acceptable to the scientific community. Therefore, results of this workshop will be carefully reviewed by EPA in making its decision on how to proceed with these particular standards.

COORDINATION WITH U.S. AND INTERNATIONAL ORGANIZATIONS IN DEVELOPMENT OF TEST STANDARDS

The Office of Toxic Substances is aware that EPA is not the only organization which has the responsibility for developing test methods. Within EPA we have joined with the Office of Pesticide Programs to form a joint work group to develop test methods which are basically consistent for the TSCA and for FIFRA. We have also joined with the three other U.S. Federal Regulatory Agencies in forming the Interagency Regulatory Liaison Group (IRLG). The IRLG committee on guidelines and standards is attempting to develop consistent standards and guidelines for EPA, FDA, CPSC, and OSHA. In addition, we are a partner or member of the Organization for Economic Cooperation and Development (OECD). The OECD will propose test standards for use by the international community in the testing of chemicals for toxic chemical control. The EPA is attempting to harmonize our test standards with the OECD.

SPECIFIC BEHAVIORAL OR NEUROTOXICOLOGICAL STANDARD

I would like now to turn my attention to specific behavioral or neurotoxicological standards that have been proposed or are under development. Under the proposed FIFRA guidlines a very minimal set of specific tests are proposed in this area. They consist of basic observations in the general toxicity tests and two specific tests for delayed neurotoxicity using hens. These are also observational tests, primarily directed to testing organophosphates and esterase inhibitors. Nothing is proposed or planned at this time by the IRLG. The OECD so far has completely avoided discussion of the needs or methods of testing for behavioral effects.

What chemicals should be tested? Of the thousands in the environment, there are probably some "sleepers" that are responsible for some of the bazaar, behavioral or neurological conditions which exist in the human population, but for which a specific cause and effect relationship has not been established. These chemicals must be identified for testing. Since Congress recognized that EPA was not the only Agency that had a concern for proper and useful data generation, it provided a provision for an interagency committee to select chemicals for EPA to test, the Interagency Testing Committee (ITC). This committee is composed for representatives of eight different Federal agencies as well as participating observers from a number of other agencies. The committee is authorized to designate up to fifty chemicals or categories of chemicals for testing at any one time. As of this time, the ITC has designated approximately 25 chemicals and categories of chemicals to EPA.

Few of the designated chemicals are proposed for testing for specific behavioral or neurotoxic effects. Several, however, are proposed for basically a general toxicity profile in which neurologic effects would be one effect to be tested for. A few of those which are proposed, for example acrylamide and arylphosphates, are already known to have neurotoxicology effects. We would welcome the review and comments by the work group participants on the 25 different chemicals or categories. If you do not have this list of chemicals, we would be happy to provide this to you.

As I indicated earlier, under Section 5, there is no provision for specific testing requirements for new chemicals, unless they fall within a category which has been proposed for testing under Section 4. Such a chemical, cannot be manufactured until it has been appropriately tested. The premanufacturing notification under Section 5 will commence 30 days after the publication of the inventory of chemicals currently in commerce. We expect that the inventory will be published in late May or early June, and therefore premanufacturing notification will begin in June or July.

We have little concept at this time as to the amount of testing that will be performed on new chemicals by the manufacturer or the type of test data that we may be provided, especially in the health area. It could be rather minimal and consist primarily of acute toxicity tests, mutagenicity tests and perhaps some subchronic toxicity testing. This will, of course, depend upon the volume of the chemical the manufacturer expects to produce and market, its release into the environment and the anticipated human exposure. In the event that the Agency does not have sufficient data provided on potential health or environmental effects and thus is unable to conduct a meaningful risk assessment, the EPA can undertake legal proceedings to prevent manufacture of the chemical.

In our attempts to impliment TSCA, we recognize that we are pushing the state of the science in developing test standards. No other legislation requires actual standards to be placed in the Code of Federal Regulations. The FIFRA requires that the Agency develop testing guidelines for the registration of pesticides. The FDA reviews pharmaceuticals and food additives but does not have an assigned responsi-

bility to provide standards for test methods to be used for the development of the toxic effects data.

I would like to mention one aspect in relation to the test methods program of the Office of Toxic Substances. We are attempting to provide for the validation of many tests that are in current use or proposed for use. A number of interagency agreements or contracts have been awarded to validate a number of test methods in certain areas, in particular that of mutagenicity or short-term tests, in acute effects and subchronic tests. We have issued a contract "Request for Proposal" to validate several of the promising tests on behavioral or neurotoxic effects. This "Request for Proposal" closes on April 30. I would encourage those of you that have the interest and scientific resources to undertake such a validation program on behavioral or neurotoxicology test methods to obtain a copy of this RFP. It is possible that some of the findings of this workshop will be useful in deciding the nature of the validation program.

ISSUES FOR CONSIDERATION BY THE WORKSHOP

I would like to conclude by proposing a list of issues that I think the workshop should consider during these next two days. These issues are directed toward the three different forms of tests which may be required under TSCA. One of these would be routine observational assessments that can be made during routine or general toxicity tests. Such observations can enhance the quality of information derived for assessment purposes. The second type of testing is the neuropathology, neurophysiology or neurochemistry examinations, which may also be conducted as a part of routine general toxicity tests or perhaps the specifics for those effects. The third type of test include those which are very specific and are designed for behavioral or neurological functions including that on the developing fetus.

The questions to be considered by the workshop are as follows: (1) How can we strengthen the acute, subchronic or chronic toxicity tests to provide the best possible indication of potential behavior of neurotoxicology effects? (2) Can and should we require a greater level of pathology examinations? How sensitive is pathology in detecting behavioral or neurotoxic effects? (3) Can we propose meaningful and validated neurochemical, physiological or neuropathology parameters which can be used in testing for neurologic or behavioral effects? (4) What existing tests for sensory, motor or cognitive effects are well enough developed and validated to be used as standards at this time? (5) Are there tests now in the research stage that need further development and validation? (6) What areas need further research on test methods? (7) How can we best group the various tests to provide for a safety assessment scheme? Should we go with a battery or a sequential scheme, and if so what would be the criteria for choosing the various tests? (For example: use pattern, structure relationships, results of prior tests, production level, etc.)? (8) Is there a logical and scientific scheme which can be used to test for certain classes of chemicals.

These are the kinds of questions OTS must address as it proceeds with its program for developing test standards under TSCA. We are hopeful that this workshop will consider these aspects as we discuss papers to be presented during the next couple of days. In looking over the agenda, we have some excellent papers on existing test methods and new test methods under development.

In closing, I would like to direct our attention to another requirement of the EPA under TSCA, we must not only consider the scientific value of the tests, but we must also consider the economic and resource limitations in applying proposed test methods to the testing of chemical substances. I would encourage discussion of these aspects along with the scientific utility of the test methods.

Test Methods for Definition of Effects of Toxic Substances on Behavior and Neuromotor Function
Neurobehavioral Toxicology, Vol. 1, Suppl. 1, p. 7. ANKHO International Inc., 1979.

Introduction and Overview

This Workshop was convened by the Environmental Protection Agency for the purpose of assessing the current capabilities of the discipline of Behavioral Toxicology for predicting neural and behavioral toxicity of environmental chemicals. Behavioral techniques can be employed to detect and establish dose-response relationships for toxicants for which the critical target is the nervous system. It has been pointed out in this meeting that behavioral tests may also detect effects upon systems other than the nervous system; i.e., establish indirect effects of toxicants, as well as make possible the identification of populations at greatest risk from a given toxicant.

Doctors Page, Tilson, Reiter, Gage and Seifter have emphasized the needs and priorities of regulatory agencies with regard to tests and methodologies for the detection and assessment of health effects. These requirements provide a challenge to behavioral scientists concerned with adapting the science of behavior to rigorous screening for behavioral toxicity. This challenge perhaps begins with the problem of definition of behavioral toxicity, which is obviously not an easy task. For convenience, a working definition probably should distinguish between acute, functional, reversible effects upon behavior, and chronic, structural damage to the nervous system which may or may not be associated with some degree of functional recovery from the primary deficit.

Other Aspects of the Challenge to Behaviorists Include

1. The need to maximize the amount of information obtainable from a behavioral test approach in order to evaluate effects upon overlapping functions, and to detect any degree of possible functional recovery. A corollary of this is the need for investigators to look for delayed effects upon behavior and for recovery from observed toxicities.

2. The need to refine estimates of the dose of toxicant to the nervous system. This may require an understanding of the metabolism of the initial exposure agent, identification of the specific neurotoxic chemical species, and establishment of the pharmacodynamic relationships involved.

3. The need to determine whether or not a dose-response relationship actually exists for all classes of neurotoxicants and what the limitations of specific behaviors are for the measurement of dose-response. There is some indication that acute or sub-chronic functional effects may plateau at low doses—perhaps due to saturation of receptors, or with the establishment of steady-state circulating and tissue reservoir levels. These considerations may be particularly important for substances administered by inhalation.

4. The need to evaluate and allow for differences between individual test animals in susceptibility to effects of a toxicant.

5. The need to consider whether enzyme induction or other effects of prior exposure to the test agent or to other compounds, treatments, etc., are affecting the outcome of the behavioral test.

6. The importance of testing for possible potentiation (or diminishment) of effects due to a given agent by the presence of other substances likely to be encountered in mixtures or adventitiously in the environment.

7. The sensitivity of the behavioral test and the relevance to the human situation may both be increased by the incorporation of a pharmacological challenge into the test.

8. The need to examine other possible treatment-behavior interactions including behavioral tolerance, sensitization-desensitization, hypersensitization, and compensation.

It is anticipated that the need of regulatory agencies for validated and comprehensive behavioral tests of nervous system function will provide clear-cut goals and objectives for behavioral scientists, and that the insights gained will result in greater coordination of research efforts—both with other behaviorists and with scientists in auxiliary disciplines such as biochemistry and pharmacology.

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Use of Discrimination Behavior for the Evaluation of Toxicants[1,2,3]

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GELLER, I., E. GAUSE, R. J. HARTMANN AND J. SEIFTER. *Use of discrimination behavior for the evaluation of toxicants.* NEUROBEHAV. TOXICOL. **1**: Suppl. 1, 9–13, 1979.—This study involved the application of discrimination behavior for the study of effects of environmental contaminants on the behavior of laboratory animals. Polybrominated biphenyl (PBB) was evaluated for effects on the acquisition and performance of a simple auditory discrimination by rats. Methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) and carbon monoxide (CO) were evaluated for effects on a delayed match-to-sample discrimination task in the juvenile baboon. All of the contaminants slowed response times and increased extra responses. These findings suggest that discrimination behavior may be of value for the evaluation of environmental contaminants for effects on the central nervous system.

Polybrominated biphenyl Ketones Carbon monoxide Environmental contaminants
Auditory discrimination Delayed match-to-sample discrimination

THIS workshop focuses upon behavioral observations which may be useful for early detection of neurotoxicity attributable to environmental agents. Assessment of potential neurotoxicity becomes a formidable task because such a large number of functions are under nervous system control and these various functions may be inhibited differentially by any given neurotoxicant. In the detection of neuroactivity, discrimination tasks are useful because they lend themselves to the simultaneous measurement of a number of CNS mediated functions. For example, the delayed match-to-sample discrimination task can be said to include associative learning, visual reproduction (short-term memory), similarities or dissimilarities in stimuli, psychomotor function and response or reaction time. The potential value of such discrimination behavior in screening for neurotoxicity is indicated by the work of Hanninen who reported in this workshop [8] that humans exposed to toluene showed marked impairment of associative learning, visual reproduction, similarities and psychomotor function. This neurotoxicity of toluene appears to be quite specific since exposure to styrene, a structurally similar compound, did not affect results of the cognitive tests but did alter psychomotor function [8].

In the study of any type of toxicity, the relevance of rodent level observations for extrapolation to humans must be continually considered. Discrimination behavioral assays may be employed in both sub-human primates and rodents offering a direct inter-species comparison of the effects of a given agent as well as optimal relevance.

We have employed discrimination behavior to evaluate the toxicity of substances that are abused through inhalation and are also encountered as environmental or potential spacecraft contaminants. The absence of any clear cut information relative to the central nervous system effects of polybrominated biphenyl provided the impetus for a study on the effects of this toxicant on the acquisition and performance of a simple discrimination task by rats.

EXPERIMENT 1

METHOD

The animals were male Holtzman, Sprague-Dawley rats, approximately three months old at the start of the experiment.

A purified and analyzed sample of hexabrominated biphenyl, obtained from NIEHS, was prepared in lecithin-liposomes suspended in saline. It was administered orally at 1 mg/kg to 12 rats Monday through Friday of each week during a one-month period for a total of 20 doses of PBB. Twelve additional rats received 20 administrations of the control vehicle during the same one-month period.

For the discrimination task, hungry rats in Skinner boxes had to select the right or left lever as correct as a function of

[1]Request for reprints should be addressed to Dr. I. Geller.

[2]Supported in part by grants and contracts from APHA no. 68-01-3859, NIDA no. DA01339, NASA no. NAS 9-14743, and NIEHS no. ES01246.

[3]The authors are indebted to Murray Hamilton for tissue analysis of PBB's.

the presence of a tone or clicker stimulus, respectively. The auditory stimuli occurred at random intervals on the average of once every two minutes (2-min VI). By making the correct choice, animals obtained milk rewards. Perfect discrimination was reflected in 100% correct responding to stimulus presentations with minimal or no responding in the absence of stimuli. Responses which occurred in the absence of stimuli reflected the general activity of the animal as well as a lack of efficiency.

Training on the discrimination task was as follows: all animals were gradually reduced to 80% of their original starting weights. They were then placed in the chambers for 1/2 hr, during which time the feeders were activated every 90 sec. On the following three days rats were placed in the chamber and given access to only the left lever. Pressing the lever would activate the clicker stimulus and produce a food reward. Animals remained in the chamber for 1/2 hr or until they made 100 responses. The right lever was then substituted for the left lever and animals received similar training in which a tone stimulus was paired with lever presses. After three days on this procedure, acquisition training for the discrimination task began. Tones or clicker stimuli occurred in a mixed order on the average of once every two minutes (2-min VI). Pressing the correct lever turned off the stimulus and activated the milk feeder. Pressing the incorrect lever simply turned off the stimulus. Response latencies for the entire session were cumulated on a running time meter.

RESULTS

PBB rats did not differ from controls with respect to accuracy on the discrimination task during the first four weeks of training. During Weeks 5–8 acquisition was more rapid for the control animals; however, the difference between PBB and control data was significant only on the eighth week of acquisition ($p<0.05$). From Week 9 onward, all rats performed at the 90% criterion level.

Throughout 24 weeks of discrimination training PBB rats were less efficient than controls in that they made many more extra responses. PBB rats also showed a trend toward longer response times throughout the experiment. Figure 1 shows these data for 18 weeks of training. Averaged weekly response time for PBB (solid lines) or control animals (broken lines) indicate PBB animals generally were slower to respond to either tone or clicker stimuli. The effect is most striking for response time measured on the right lever.

Ten months after the last PBB administration rats were sacrificed and analyzed for PBB levels by electron capture gas liquid chromatography. PBB was found in whole brain in concentrations ranging from 0.038 to 0.40 μg/g wet weight. PBB was also found in plasma in concentrations ranging from 0.135 to 0.372 μg/ml.

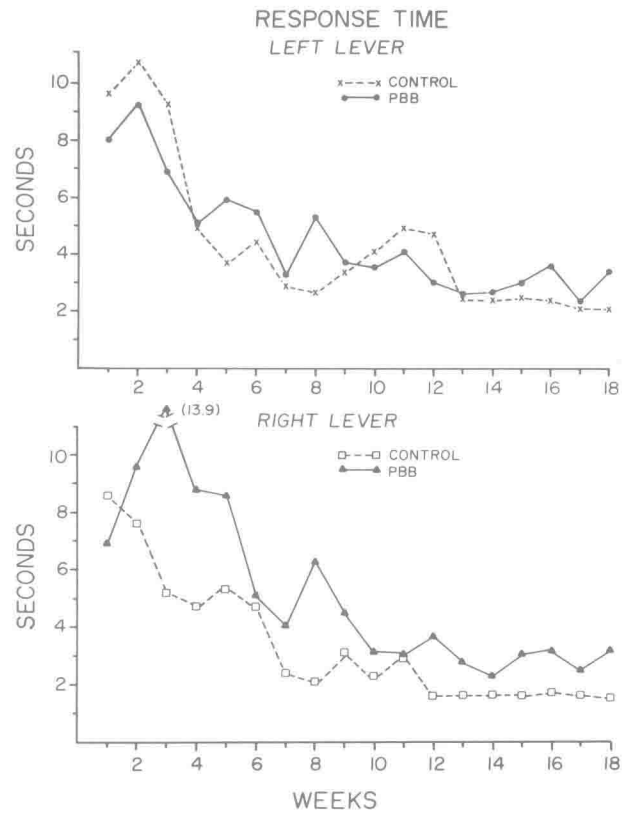

FIG. 1. Effect of polybrominated biphenyl on response time of the rat.

EXPERIMENT 2

METHOD

A match-to-sample discrimination task was used for the baboon and the toxicants were gases administered through inhalation in a flow-thru system.

The behavioral test chambers and gas exposure chambers have been previously described [7]. Two large stainless steel chambers equipped with a walk-in air lock were used to conduct the exposure. The chambers measured approximately nine feet high and nine feet in diameter. Juvenile male baboons approximately two years of age were housed in behavioral test chambers which were maintained in the large exposure chambers. The behavioral test chambers were designed so that an intelligence panel could be slipped down between the outside wall of the cage and the baboon. The intelligence panel was equipped with a row of three translucent discs which served as levers. Under the appropriate experimental conditions, pressing either side disc produced a banana pellet reward. Experimental sessions of two-hour duration were conducted on Monday through Friday of each week.

When the session timer was activated, a variable interval (VI) tape was set in motion. The tape programmed the occurrence of a stimulus on the center lever on the average of once every three minutes. The VI tape was inoperative during each trial which began with the illumination of one of the stimuli, the probe stimulus on the center lever. The stimulus was terminated after a 30-sec period or by a response on the lever. Termination of the stimulus activated a timer for a two-minute delay interval. At the end of the delay interval, stimuli appeared on both levers adjacent to the center lever. The correct matching stimulus was varied between these two levers in a mixed order. A response on the correct lever, where the stimulus matched the center lever stimulus, terminated the stimuli, activated the feeder and produced a banana pellet reward. Responses on the incorrect lever simply terminated the stimuli and again set the VI tape in motion.

A record was kept of the number of probe stimuli presented during each 15 min segment of a two-hr session, the numbers of correct matching responses on right and left levers and the number of incorrect responses. A record was also kept of any extra responses that may have occurred on the three levers when the stimuli were not activated or during the delay interval. The time it took the animal to press the lever after a stimulus was activated was also measured (response time). After the baboons were trained to 90–100% efficiency on the discrimination task, the exposure phase of the study was begun.

The animals were exposed to methyl ethyl ketone (MEK) or methyl isobutyl ketone (MIBK). Exposure was by means of the vapor saturation technique [3]. For the vapor saturation method, air is bubbled through a gas washing bottle containing the liquid to be vaporized. In passing through the liquid, the air becomes saturated with vapor which is then directed to the air intake ducts of the exposure chamber. Changing the flowrate with the fine metering valve or changing the temperature of the constant temperature bath allows one to produce a range of pollutant concentrations in the exposure chamber. The technique is simple and works well for substances that are liquids at room temperature. A Hewlett-Packard gas chromatograph, modified for automatic sampling was employed. This allowed for automatic sampling, quantitation and recording of pollutant concentrations in the exposure chamber.

The animals were also exposed to carbon monoxide (CO). The exposure atmospheres for the carbon monoxide studies were produced using compressed gas cylinders of CO obtained in 99.5% purity. The correct amount of carbon monoxide was introduced into the chamber by means of a calibrated flowmeter and a fine metering valve. Samples of chamber air were withdrawn with 1 ml gas tight syringe and analyzed on a gas chromatograph. The GC uses the principle of catalytic conversion to hydrogenate CO to methane which is detected with a conventional flame ionization detector. Samples were analyzed on the average of once every 10 min. The concentration was determined by a comparison of the detector response for a chamber air sample with the detector response for a series of standard samples. With these techniques, exposure chamber atmospheres were maintained within 10% of the desired value.

Animals were exposed to the ketones for 24 hr per day during a seven-day period. They were exposed to 100 ppm MEK, 50 ppm MIBK or to a combination of MEK or MIBK at the same concentrations. These concentrations are half the established threshold limit values [5]. While two animals in one of the chambers were being exposed to a contaminant atmosphere, the animals in the other chamber served as controls and were exposed to clean air during the same period. Thus, not only did other animals serve as controls, but each animal served as its own control in that exposure data could be compared with data obtained pre- and post-exposure time.

Animals were exposed to 25 ppm or 50 ppm CO for six hr per day during a one-week period while the two-hr behavioral test was conducted in the morning or afternoon. Thus, behavioral testing took place during the first two hr of CO exposure in the morning or in the afternoon after the animals had already been exposed for four hr.

RESULTS

For the ketones, performance on the match-to-sample task was not impaired under any of the three experimental conditions. However, response times or numbers of responses during the delay periods were affected by the gases in the four test animals.

Figure 2 shows these effects in two baboons. The solid lines represent exposure data and the broken lines, control

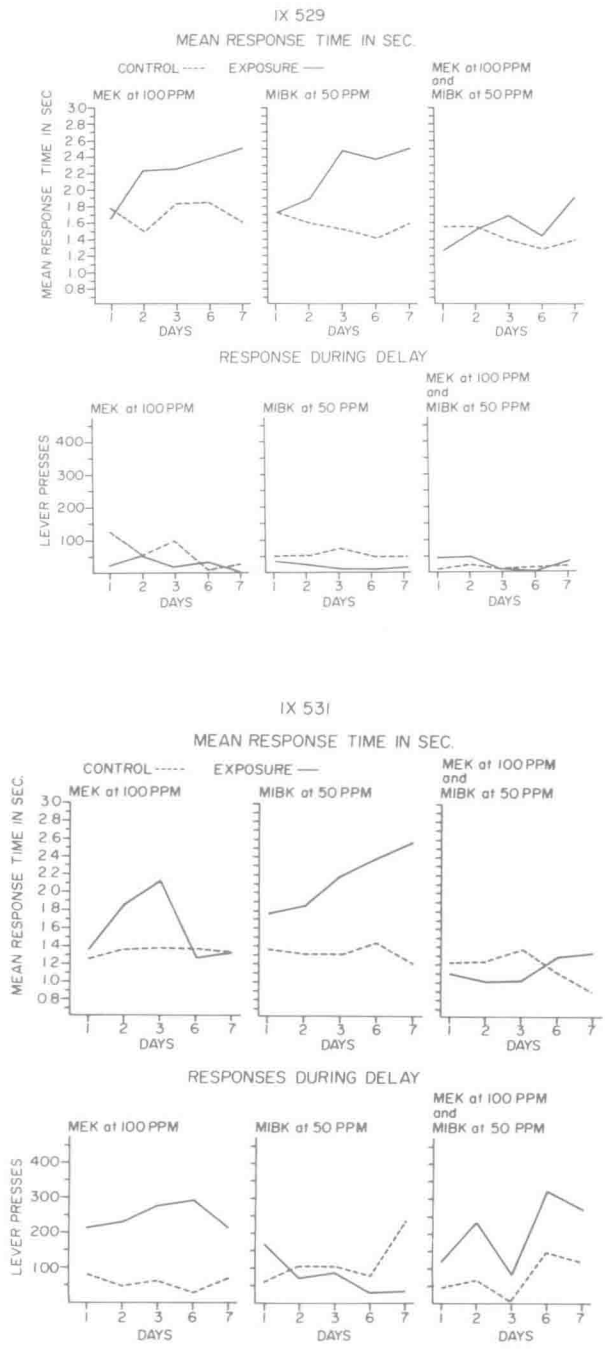

FIG. 2. Effect of seven-day exposures to 100 ppm MEK or 50 ppm MIBK, administered alone or in combination on match-to-sample behavior of baboons. Control data are represented by broken lines and exposure data by solid lines.

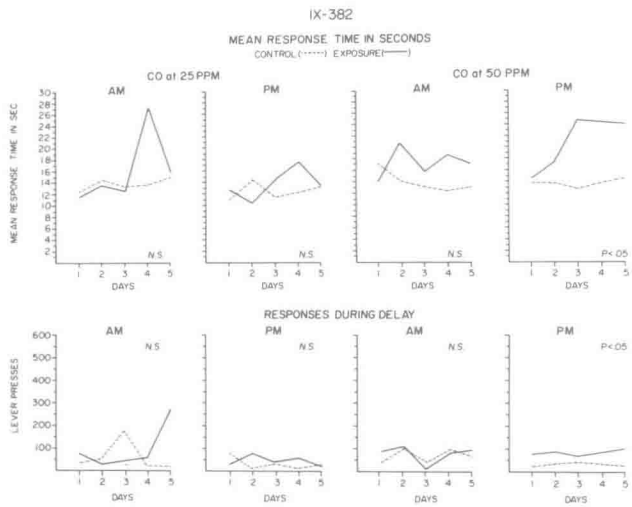

FIG. 3. Effects of six-hr daily exposure to carbon monoxide during a five-day period. Broken lines represent control data while solid lines represent exposure data.

data, obtained during a seven-day, pre-exposure period. For Baboon 529, mean response time increased above control levels under 100 ppm MEK during each of five behavioral sessions. The same was true for MIBK at 50 ppm. However, under a combination of MEK and MIBK at the same concentrations, the exposure data approximated that of the pre-exposure control. Mean response time for Baboon 531 increased gradually under 100 ppm MEK during the first three exposure days. On Days 6 and 7 the data were like that of controls. Mean response time under 50 ppm MIBK increased throughout the week of exposure. Again a combination of 100 ppm MEK and 50 ppm MIBK produced data similar to controls.

Responses during the delay intervals were like that of controls for Baboon 529, while for Baboon 531 there occurred a large increase under MEK, little or no effect under MIBK and an increase with the combined MEK, MIBK exposure.

For CO, a slight impairment of discrimination occurred at 50 ppm; animals exposed to this concentration of CO occasionally made a mistake.

Data typically obtained for response time latencies or responses during the delay are shown in Fig. 3; the broken lines in the figure represent control data averaged for each of five pre-exposure days and the solid lines represent data for five exposure days. At 25 ppm, CO produced a slowing of response time on Day 4 of the morning and afternoon exposures. These differences between exposed and control animals were not significant. Responses during the delay intervals did not change significantly under 25 ppm CO. The 50 ppm CO exposure produced a slowing of response time after Day 1 which persisted throughout the five-day period. This effect was significant for the afternoon animal who had already been exposed four hr each day when behavioral testing began. Responses during the delay interval increased significantly only for the afternoon animal during exposure to 50 ppm CO.

EXPERIMENTS 1 AND 2

DISCUSSION

Discrimination tasks have been used for the study of a number of psychoactive agents [6, 9, 10], and several rat studies have indicated that discrimination behavior may be of value for the study of certain central nervous system (CNS) active compounds [6,9]. It would appear that the application of discrimination behavior in a sub-human primate as well as in the rat should be a valuable technique of the relatively new field of behavioral toxicology. We have described here a simple discrimination task for the evaluation of toxicants in rats and a match-to-simple discrimination task for the evaluation of toxicants in young baboons. Application of these tasks to the study of effects of PBBs in rats, and to the study of effects of inhaled ketone vapors in baboons, is illustrated.

Rats treated with 1 mg/kg PBB were like controls with respect to accuracy on the discrimination tasks, however, extra responses and response latencies generally increased, thereby reducing the animal's efficiency.

Similarly, MEK or MIBK administered chronically at half the TLV over a seven-day period did not impair the baboons' ability to discriminate but did alter response latencies and extra responses during the delay intervals. The combinations of MEK and MIBK produced less effect on response latencies than did either one of the individual gases. Since the animals were esposed to the single gases prior to being exposed to the mixtures, it is possible that monooxygenases were induced in liver or extra-hepatic tissues that affected metabolism of the compounds on subsequent exposure, or that simultaneous inhalation of one compound affected the metabolism of the other compound. A loss of effect on response latency which occurred on the sixth and seventh day for two animals exposed to MEK alone might also be accounted for in terms of enzyme induction which increased metabolism of the inhaled gas.

The effects noted here with MEK and MIBK and the lessening of effect with a combination of the two vapors is of special interest. Both MEK and MIBK have been considered to be non-neurotoxic whereas methyl n-butyl ketone (MnBK), through the action of its metabolites, has been found to produce peripheral neuropathy, which is potentiated by simultaneous inhalation of MEK [1, 11, 12]. However, the potential CNS toxicity of MnBK has not been considered. The potentiation of the peripheral neurotoxicity of MnBK by MEK has also been associated with MEK-induced stimulation of microsomal enzyme activities; this effect is also manifested as a decrease in hexobarbital-induced sleep times [4]. However, these studies involved much higher vapor concentrations and longer exposure times than employed in the present studies.

The behavioral effects produced by MEK or MIBK at half the TLV concentrations indicate that the solvents are acting on the central nervous system. If the ketones rather than their metabolites are the neuroactive forms, enhanced metabolism might account for the observed loss of central nervous system effects in these studies. The consequences of human exposure to sub-TLV concentrations of these ketones should be evaluated.

Carbon monoxide increased extra responses during the delay interval while its greatest effect was to slow reaction time. Theodore *et al.* [13] reported a similar slowing of reaction times in monkeys exposed to almost twice the TLV of