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Behavioral Toxicology of Carbon Disulfide and Toluene

by Bernard Weiss,* Ronald W. Wood,* and David A. Macys*

Organic solvents are pervasive in the communal and industrial environments. Although many are potent central nervous system agents, clearly delineated behavioral effects have played only a minor role in the formulation of exposure standards. A comprehensive behavioral pharmacology and toxicology of these compounds is one aim of the US/USSR collaboration. The current report describes some actions of carbon disulfide and toluene. Earlier data about the actions of carbon disulfide on pigeon operant performance indicated disruption of schedule-controlled key-pecking. Primate data are now described from a situation designed to determine aversive thresholds to electrical stimulation. Effective concentrations of carbon disulfide produced both a rise in the amount of electric shock tolerated and a diminution of the response force exerted by the monkeys. In experiments with toluene, pigeons were shown to elevate key-pecking rate in an operant situation at certain concentrations. Toluene also was studied for its capacity to maintain self-administration in the same way as drugs of abuse. Monkeys worked to gain access to toluene vapor just as they work for opiates or amphetamines. The current experiments demonstrate how comprehensive the range of behavioral toxicology needs to be to deal with environmental health issues.

Despite their pervasiveness in the industrial and community environments and their ability to produce pronounced central nervous system effects, organic solvents are rarely studied like other pharmacologically active substances. Rigorous laboratory investigations constitute a small portion of the industrial hygiene literature. With rare exceptions, worker complaints, clinical experience, and gross toxicity comprise the basis of most current exposure limit values. Since subtle behavioral effects frequently represent the earliest toxic signs of exposure, the environmental health sciences need to evaluate the toxic potential of organic solvents by behavioral assays. Even in the Soviet Union, where neurotoxicology is relatively well established (*1*), a comprehensive data base for organic solvents does not yet exist.

Our role in the collaborative program with the USSR began with studies of carbon disulfide (CS_2), and focused on the behavior of the pigeon (*2, 3*). We employed operant conditioning techniques (*4*) developed by behavioral pharmacologists because such techniques are sensitive to many centrally active agents. This report describes further aspects of our continuing program, which now includes an additional agent, toluene, and a nonhuman primate species, *Saimiri sciureus* (the squirrel monkey).

Vapor Generation Technique

Precisely controlled and monitored delivery systems are critical if meaningful statements about the parameters of exposure are to be made. Liquid solvent is placed in a standard gas washing bottle (bubbler); compressed air, which is filtered to remove oil and particulates, is metered through the solvent by an upstream rotameter. The vapor then is mixed with dilution air and enters the exposure chamber. A single-beam infrared spectrophotometer (Wilks Scientific Miran-1A portable gas analyzer, equipped with a 1-m, 350 ml nickel-and monel-plated cell with NaCl or AgBr windows) monitors concentrations continuously. Samples are drawn from the chambers and through the spectrophotometer by a pump; sampling rate is controlled by a rotameter at the pump downstream from the spectrophotometer. Absorbance readings

* Department of Radiation Biology and Biophysics, University of Rochester Medical Center, Rochester, New York 14642.

are provided by the spectrophotometer and a strip chart recording made to monitor stability. Multiple exposure chambers are monitored by sequentially sampling each chamber through a solenoid-activated valve manifold. Air drawn through activated carbon provides a zero reference for the system. Exhaust from the pump and the chambers is ducted to a standard laboratory hood.

Important determinants of specific exposure situations included: the species; physical and chemical properties of the solvent, particularly the volatility, flammability, and the propensity for surface adsorption; exposure concentration and duration; volume of exposure chamber; range of acceptable flow rates through either the bubbler or the chamber; the integrity of the chamber seal; and the potential exposure hazard to laboratory personnel. The above parameters determined the selection of: vapor generation efficiency of the bubbler, rotometer range and accuracy; presence of an ice bath; proximity of vapor generator to exposure chamber and its ventilation; types of tubing, connectors and sealants; method of exhausting exposure chambers; and type of exhaust ventilation. For example, the CS_2 system required very low flow (0.3–100 ml/min) rotometers, plain-tip bubblers, and ice baths to generate low and stable vapor concentrations for long periods of time (100 ppm for 18 hr) with minimum attention. Teflon and/or polypropylene tubing minimized adsorptive phenomena in the vapor delivery system, while a special nickel alloy plating was used in the spectrophotometer. Screw-type hose clamps and caulking compound helped prevent leaks. The exposure chamber was maintained at a negative pressure with respect to its enclosure; the enclosure was, in turn, maintained at a negative pressure with respect to the room. The room itself is at a negative pressure with respect to the rest of the laboratory.

Methods and Results

Effects of Toluene on a "Counting-Like" Behavior in the Pigeon

An organism's behavior can generate internal stimuli that, like external stimuli, can come to exert control over the behavior. The differential sensitivity to pharmacologic or toxicologic insult of performances controlled by these two classes of stimuli has been examined within the context of a single schedule of reinforcement, the fixed consecutive number (FCN) schedule (*5*). Investigators in this and other laboratories have demonstrated its sensitivity to drugs with significant CNS actions (*6–8*). It also is sensitive to carbon disulfide (*2, 3*). In the current experiments, the FCN schedule was arranged as follows. When a pigeon emitted 20 or more consecutive responses on the left key before switching to the right key of a two-key chamber (FCN 20), a feeder containing grain rose into position following the right key response. If the right key was pecked prematurely, that is, before the 20th peck on the left key, the response requirement was reset and the chamber illumination extinguished for 5 sec. In this situation, the subject's behavior presumably is under the discriminative control of a property of its own prior behavior, namely, numerosity.

Figure 1 shows representative cumulative records of this performance after 4-hr exposures to toluene in concentrations ranging from 0 to 3200 ppm. All toluene concentrations increased the rate of left key

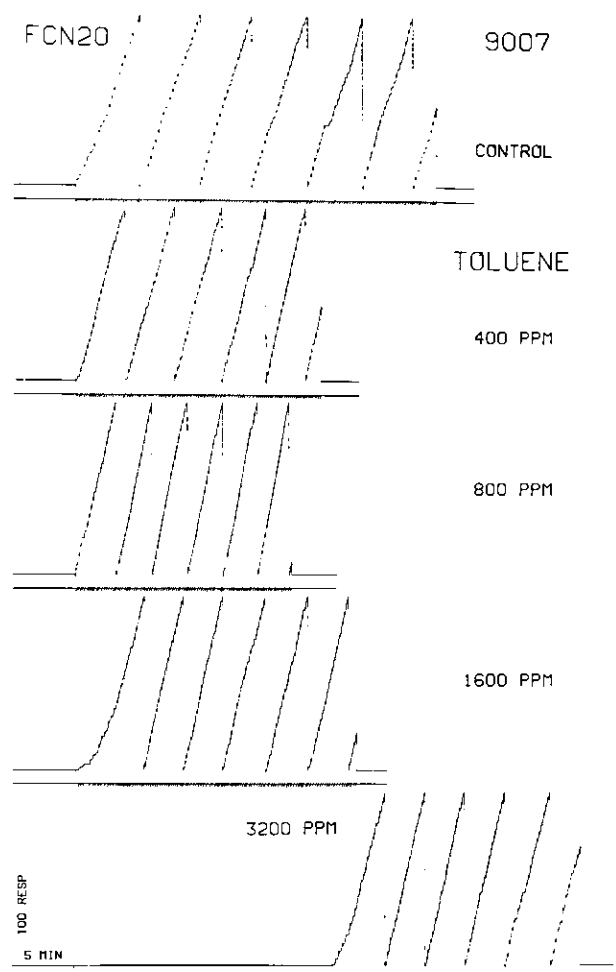

FIGURE 1. Cumulative records of FCN 20 performance after 4-hr exposures to various concentrations of toluene. Time runs from left to right; each left key response moves the pen upwards. The pen deflects with reinforcer delivery. The lower pen deflects with each right key response. The session terminated after 100 reinforcements.

responding (note steeper slope). The highest concentration (3200 ppm) produced a long period without responding at the beginning of the session. The subject was grossly ataxic when inserted in the chamber, and responding started 28 min later. Figure 2 presents several derived measures of this performance. Post-reinforcement interval (PRI) is the time between the end of the feeder cycle and the first peck on the left key; switch time is the latency from the last peck on the left key to the reinforced peck on the right key; left key interresponse time (IRT), is the interval between successive pecks; reinforced runs is the proportion of left-right sequences reinforced; run length is the number of consecutive pecks on the left key before a switch.

Two temporal measures, post-reinforcement interval and switch time, displayed a U-shaped concentration-effect function with toluene exposure, first falling, then rising with concentration. Concomitantly, overall response rates increased at lower concentrations and decreased at higher ones. The peak effect occurred at 800 ppm. The sensitivity displayed by these measures probably is attributable to the absence of a ceiling effect: their relatively long durations provided extensive margins of modification. Left key interresponse times could not be shortened significantly because of inherent physical limitations. Percent of runs reinforced (a reflection of the run length) increased with toluene exposure, although not monotonically. This was the first time a stretching of run lengths has been observed; we subsequently observed such an effect after ethanol (V. G. Laties, personal communication); barbiturates and minor tranquilizers are yet to be studied in this preparation.

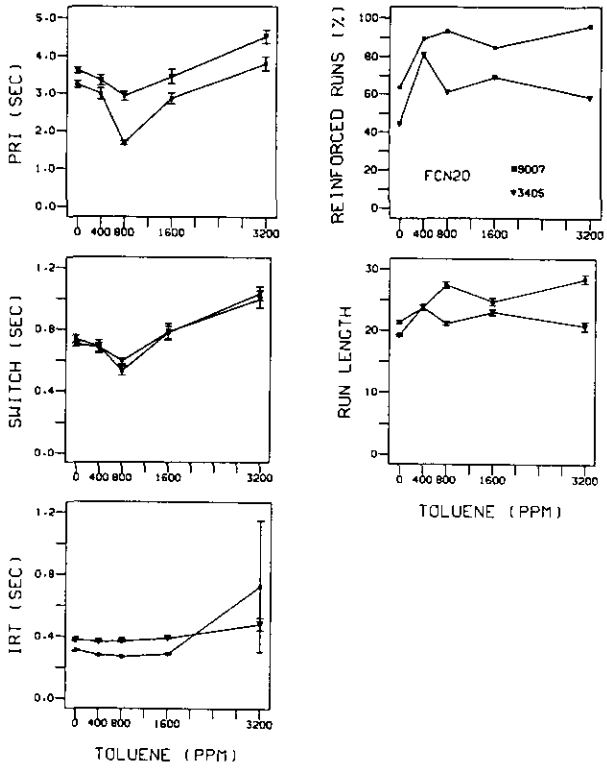

FIGURE 2. Derived measures of FCN 20 performance as a function of toluene concentration (4-hr exposure duration) for birds 9007 and 3405. Plotted are the means ± S.E. Zero points derived from four sessions; other observations derived from a single exposure at each concentration, obtained in an increasing series (2/week). No day-after effects were observed.

Effects of CS_2 on Aversive Threshold in the Squirrel Monkey

Neuromuscular stimulation by shock electrodes applied to the feet (*9*) is a common assay for toxicity in the USSR. Avoidance performance maintained by electric shock is a frequently employed method of behavioral assessment in the West. The titration schedule (*10*), unlike these intermittent stimulus paradigms, provides a continuous assessment of aversiveness. In the prototypical arrangement, shock intensity increases incrementally at a constant rate. When the subject emits a response, shock intensity is reduced by a specific decrement. Titration performance is particularly sensitive to analgesic drugs, e.g., morphine (*11*). Such drugs elevate the aversive threshold, i.e., the level to which the shock is permitted to rise.

In the experiments reported here, the squirrel monkey subjects sat in a cockpit, restrained at the waist. The monkey faced a T-shaped Lucite bar fixed to a strain gauge that could measure up to 1 kg (Fig. 3). A computer-controlled, constant current shock stimulator delivered the aversive stimulus to electrodes placed on the tail and foot. Stimulator output ranged from 0 to 3 mA. Strain gauge output was amplified by a polygraph and fed to the analog-to-digital inputs of a digital computer. A large force requirement (300 g) enhanced the sensitivity of the preparation to toxicologic insult. Shock level rose by 2% of the total range (60 μA/step) each time an increment was programmed (every 2 sec) and fell by the same amount after each response. The bar had to be released (the force falling to less than 5 g for 100 msec) to initiate a new response; continued application of such a force did not further lower the shock. All schedule parameters were entered in the computer program that controlled the experiment.

A 2-hr exposure to approximately 600 ppm (1.87 mg/l.) produced a striking effect in one monkey and a marked, but more subtle change in the second.

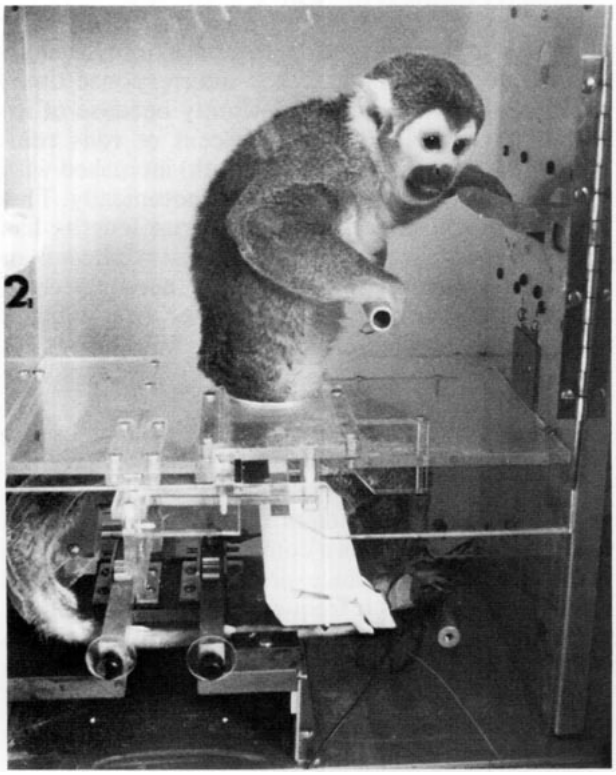

FIGURE 3. Squirrel monkey (*S. sciureus*) in restraint device employed for titration schedule performance. The plastic bar grasped by the monkey fits over a strain gauge whose output is amplified and fed to the analog/digital inputs of a computer. The output of a computer-controlled shock stimulator is delivered to the tail and foot.

The performance of the first monkey appears in Figure 4. Control performance (left) is remarkably stable. The aversive threshold rose gradually for the first few minutes, then remained within narrow limits for the rest of the hour session. The undulating character of the aversive threshold arises from the tendency of the monkey to wait until the shock rises by several steps before emitting a train of responses. CS_2 radically altered this pattern. For about 30 min after exposure, the monkey responded erratically, tolerating a higher shock level than under control conditions. Two phenomena appear to be responsible for the elevated threshold. The first is the intrusion of long gaps without responding. The second is the inadequate force exerted when the monkey did perform; responses less than 300 g did not reduce the shock. As the records reveal, most responses during this period fell below the criterion, thereby leaving the shock elevated and occasioning the higher response rate.

A further consequence of the exposure is seen in the record segments for the last 30 min of the session. Although response forces during this period met the criterion as often as during control sessions, the aversive threshold remained elevated beyond control values, suggesting an anesthetic or analgesic effect. Such an effect was duplicated by the performance of the second monkey. Although this subject did not display an inability to meet the force criterion, it maintained a shock level 50% higher than its typical control value. Additional experiments with lower exposure concentrations over longer periods produced equivalent effects. The monkey whose performance is depicted in Figure 4 displayed a similar response to an exposure of 18 hr at 70 ppm. The highest concentration required to produce such an effect in a group of four trained monkeys was 200 ppm.

Toluene "Sniffing" by the Squirrel Monkey

Substances consistently abused by humans are also self-administered by nonhuman primates (*12*, *13*). Humans self-administer a wide variety of organic solvents and volatile anesthetics by inhalation (*14–17*). We have recently reported the development of a nonhuman primate model of this type of substance abuse (*18*). Squirrel monkeys were trained to push a button which initiated the flow of nitrous oxide (N_2O) through a helmet enclosing the monkey's head. Control experiments demonstrated that it was an effective reinforcer, very much like food, water, or intravenous drugs. Recent work from this laboratory indicates that toluene also is a reinforcer. Squirrel monkeys previously trained to self-administer N_2O were placed in an apparatus (Fig. 5) in which 15 sec deliveries of toluene vapor were made contingent upon a single response (fixed ratio 1). The frequency with which monkeys self-administered toluene vapor was an inverted U-shaped function of vapor concentration (0.056 to 1.0%). The highest average rate observed at any concentration was 141/hr at 0.1%. Figure 6 shows representative cumulative records of this performance when concentration was varied between one hour self-administration sessions. Response rates were stable and reinforcer deliveries rather evenly spaced when toluene was delivered contingent upon a response. Responding occurred irregularly and in bursts when toluene was not available.

Future Directions

Selection of Behavioral Assays

The diversity of techniques described here is a deliberate choice. We realize that such diversity may be puzzling to specialists unacquainted with behavioral toxicology, but it represents a major

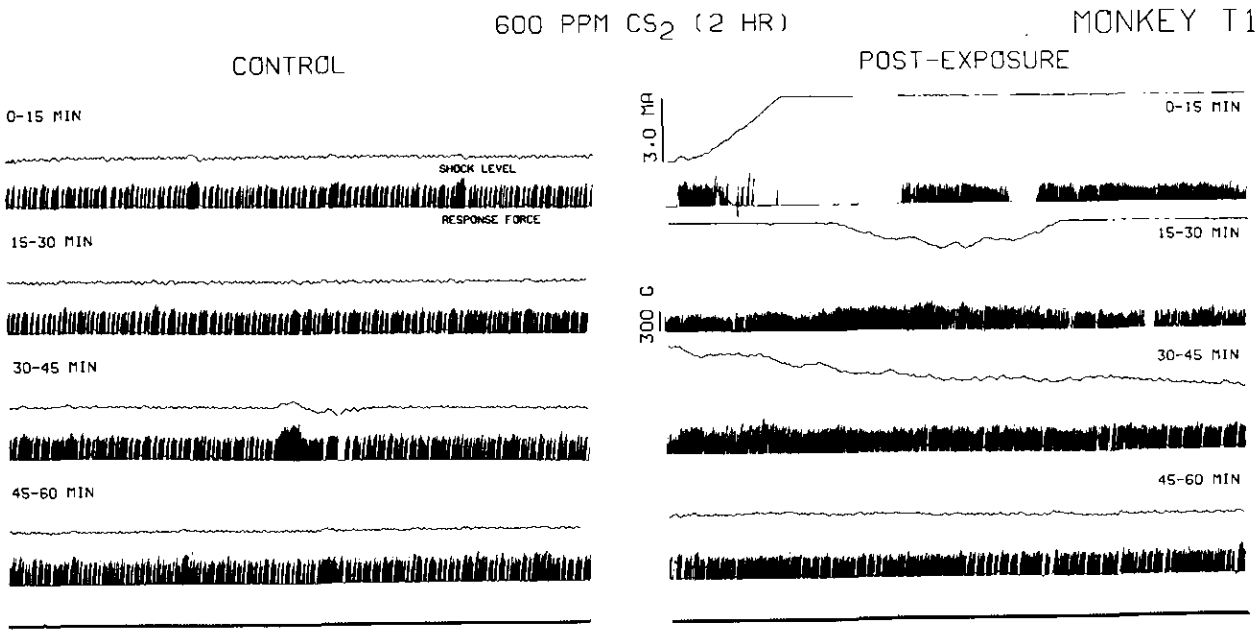

FIGURE 4. Aversive thresholds after exposure to 600 ppm carbon disulfide for 2 hr. Each pair of records traces out the shock level tolerated by the monkey and the response force exerted on the T-shaped lever. Under control conditions, shock level remained relatively constant at close to 0.3 mA. Typically, the monkey allowed the shock to rise by a few small steps, each equal to 2% of the total range, and then reduced it by emitting a train of responses on the lever. Such a pattern is reflected by the clustering discernible in the response force record. During the first 30 min after exposure, the shock was allowed to rise to the maximum (3 mA) and to remain there for most of that period. Although many responses were emitted, they lacked sufficient force to meet the 300-g criterion. After approximately 30 min, the shock was gradually reduced, and by 45 min after exposure had attained a relatively stable level, although elevated above control values. Only toward the end of the 1-hr period, however, did the force record begin to display the characteristics that can be seen on the control record, namely, above-criterion responses emitted in clumps.

strength of this discipline (*19*). Our ability to pose problems from several different vantage points enables us to specify the form and character of a behavioral impairment, an ability more illuminating than a simple decision about the presence of toxicity. Operant behavior of laboratory animals may not enable us directly to set exposure standards for humans, but how else are we to decide on which behavioral criteria to employ for such standards? Data accumulated in this laboratory clearly demonstrate that certain types of behavior are more susceptible to toxicologic impairment than others, and that agents differ in the pattern of effects they create (*2, 3, 6, 11*). Such an outcome is to be expected; it is probably the best-established principle of behavioral pharmacology. More than 20 years ago, Dews (*20*) observed that patterns of behavior generated by different schedules of reinforcement are differentially sensitive to pharmacologic insult. Later, Kelleher and Morse (*21*) demonstrated that the effects of drugs depended upon these contingencies of reinforcement and the ongoing pattern of behavior thereby generated, rather than on the nature of the reinforcing event (food delivery or shock termination). McKearney (*22*) has qualified these observations by demonstrating that the nature of the reinforcing event can determine the effect of drugs on punished performance. The behavioral effects of a drug or poison cannot be characterized independently of the context in which the behavior occurs. Indeed, the behavioral effects of an agent are best understood in behavioral terms; associated changes in the structure and function of the nervous system may be of interest, but changes at this level of analysis cannot reliably explain behavioral effects (*23, 24*). A significant component of behavioral toxicology must continue to be the calibration of methods with reference substances, often drugs, so that the behavioral determinants of toxicity are not relegated to an ancillary role (*25*).

Primates as Subjects

A choice to use a nonhuman primate species for a toxicologic experiment should consider not only the care and expense entailed, but also the pressures threatening these species in their shrinking natural habitats. Their phylogenetic proximity to humans appears especially important for assays of central nervous system toxicity. We are confident in ex-

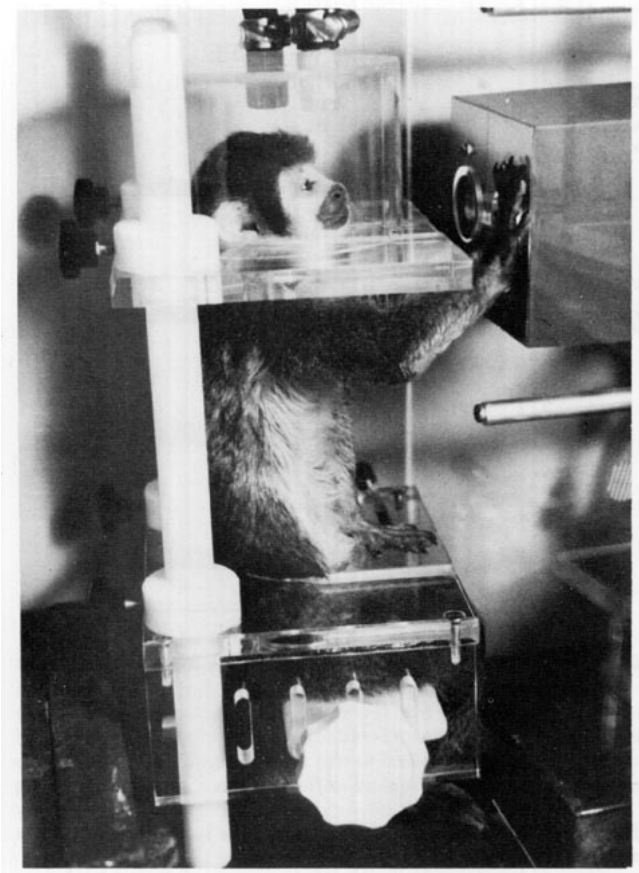

FIGURE 5. Squirrel monkey seated in toluene self-administration apparatus. Subject is pushing button that produces a 15-sec infusion of toluene vapor into the helmet resting on the neck restraint plate of the chair.

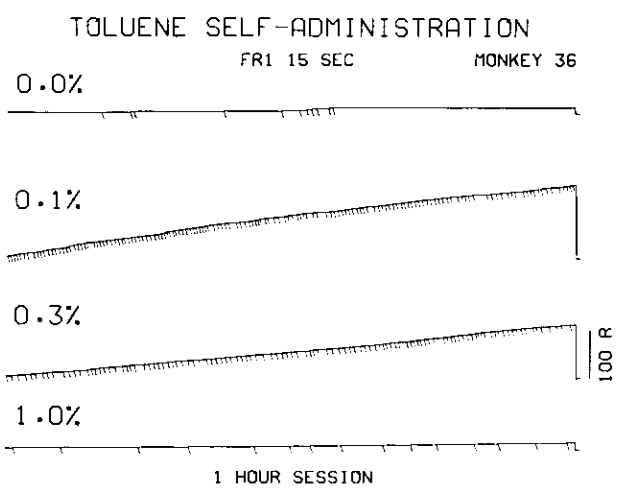

FIGURE 6. Cumulative records of monkey 36, illustrating fixed ratio 1 performance maintained by 15 sec deliveries of toluene. Toluene concentrations are indicated at the left of each cumulative record. Session duration was 1 hr. Note irregular responding in the absence of toluene; response and reinforcement rates at these concentrations were inversely related to concentration.

trapolating from nonhuman primates to humans because of our previous experience with methylmercury (*26*) and neuroleptic drugs such as haloperidol (*27*). Nonhuman primates play a key role in research on substance abuse; monkeys will self-administer any substance abused consistently by humans, including organic solvent vapors. Although the most attention and concern has been directed at the abuse of inhalants by youth, this pattern of substance abuse is known to be practiced discreetly by adults in occupational settings (*28–30*). Pure toluene has no known gross toxicity (*31*); however, when industrial grades of toluene or commercial products such as spray paint are abused, toluene serves as the reinforcing vehicle for the ingestion of more toxic substances. The intentional inhalation of halogenated hydrocarbons, such as trichloroethylene, chloroform, halothane, and vinyl chloride, may expose the sniffer to delayed forms of toxicity ranging from liver damage to cancer.

Information Exchange

A persistent problem in the US-USSR exchange must also be addressed. If the joint program in behavioral toxicology is to be a continuing and successful venture, the exchange of literature must be facilitated. A substantial body of literature remains unindexed and inaccessible to Western information retrieval systems. Repeated attempts to obtain cited reports have been frustrated either because documents are nowhere to be found in the continental US or because technical translation services are not readily available. Certainly, all archival Soviet journals should be included in the collection of the National Library of Medicine. The Toxic Substances Control Act (PL 94-469, Sec. 10) mandates centralized indexing of toxicologic information; the Soviet efforts may constitute an important contribution to this data base. When an investigator in the environmental health sciences needs a pertinent document, the National Library of Medicine should procure and translate the document at the request of NIEHS or EPA. The translation should then be catalogued. Of particular interest and value are reports filed at research institute libraries in the USSR. Numerous problems confront the foreign investigator wishing access to such sources. Such institute reports contain both data and detailed descriptions of techniques which permit not only the direct scrutiny of experimental results but also the replication of experimental procedures.

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