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Behavioral Assays in
Environmental Toxicology

MASTER

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At a meeting that features the theme of in vitro test systems, behavioral assays may strike you as a primitive anomaly. Yet, environmental toxicology is still too permeated by questions about how the whole organism functions to abandon intact animals as test systems (Weiss, 1978). Although the threat of cancer may foment the most intense anxiety about toxic chemicals, the public also exhibits a simmering disquiet about other adverse effects. The volatile argument provoked by assertions that food additives are implicated in the etiology of hyperkinesis is neither an isolated nor a transient phenomenon. It exemplifies, in fact, one of the forces sweeping behavior into the orbit of the environmental health sciences.

Behavior does not participate as a single entity or discipline. It ranges across the total spectrum of functional toxicity, from tenuous subjective complaints to subtle sensory and motor disturbances demanding advanced instrumentation for their evaluation. I will discuss three facets of behavioral toxicology that illustrate its breadth of interests and potential contributions.

Psychophysics

Disorders of sensation often serve as sentinels of adverse effects localized in the nervous system. Although such disorders may arise from detectable neuropathology, which represents an end stage, they also may express a functional disruption of unknown, unspecified, or undetectable mechanisms. Even clearly defined neuropathology, moreover, may not contribute to issuing exposure standards. How much of a safety factor is required for minimum risk? How much is desirable? How much is practical?

Psychophysics in the discipline devoted to the relationship between sensory processes and physical parameters and is the oldest branch of experimental psychology. Its importance to Behavioral Toxicology lies in its ability to provide precise assessments of sensory function. Clinical neurology also evaluates sensory functions, but from another point of view. Clinical examinations focus on overt disease. Environmental standards, in contrast, aspire to prevent any degree of damage, no matter how superficially inconsequential.

Vision provides convincing examples of the role of psychophysical assessment because impairment of visual function often reveals early evidence of neurotoxicity (Merigan and Weiss, in press). Methylmercury furnishes a striking example. In the early 1950s, fishermen and their families from Minamata, a fishing village on the southernmost Japanese island of Kyushu, began to fall victim to a neurological disease eventually diagnosed as methylmercury poisoning. Peripheral constriction of the visual field was a characteristic accompaniment and aftermath of the intoxication, and, in fact, one of the symptoms (Table I) on which a diagnosis is based. About 10 years later, a similar episode occurred at Niigata, which is located on the island of Honshu. In both locations, the source of the poison was a factory that employed mercury as a catalyst in the production of aldehyde, dumped it into local bodies of water as waste, and contaminated the seafood eaten by local inhabitants. The Niigata episode, because Japanese investigators already had been prepared by Minamata, confirmed clearly the sensitivity of vision as an index of excessive methylmercury ingestion.

The discovery of elevated methylmercury concentrations in Great Lakes fish early in 1970 provoked debates in the U.S. about the hazards of current levels. Soon after, an epidemic of poisoning, from grain treated with a methylmercury fungicide, erupted in Iraq. These coupled events stimulated us to clarify both the course and features of methylmercury intoxication, especially the relationships among dietary level, exposure duration, blood level, brain pathology, and sensory function. We chose vision as an index of damage because the clinical and epidemiological data indicated it to be the most stable and enduring functional deficit, at least in adults and older children.

Neuropsychology and psychophysics helped determine our choice of approach. Neuropsychology told us that we had to perform our experiments on non-human primates, because only primates possess a visual system analogous to humans in both structure and function. It also told us that vision in dim light (scotopic vision) was more likely to prove sensitive than vision at high luminance (photopic vision) because the peripheral visual field, whose function is impaired by methylmercury, subserves scotopic function. These expectations were confirmed in a series of experiments with different dose levels and durations of exposure (Evans, Garman and Weiss, 1977). Fig. 1 shows the performance of monkeys (*M. arctoides*) trained to perform a complex visual discrimination requiring them to identify and select a geometric form projected onto the face of a response key. These were revealing experiments. They indicated that visual deficits like those induced by methylmercury could be detected in intact organisms by functional tests, that they were related to exposure parameters, and that they could be correlated with morphological damage.

These findings have now set the stage for a more extensive exploration of visual function, embracing not only methylmercury, but methanol, carbon disulfide, and other important environmental agents. The impetus came not only from the still unresolved questions about methylmercury, but from newer concepts of visual function. These have created novel approaches in visual science. It is now widely accepted that the visual system is a frequency-sensitive system--that visual information is encoded as frequency information. The frequency domain extends over two dimensions--spatial and temporal.

Many of you know that temporal frequency discriminative capacity is tested by flickering a visual target and determining the frequency at which the flicker fuses into steady illumination. This value is called the critical frequency of fusion (CFF). The contemporary view of the visual system as a frequency (or Fourier) analyzer makes the conventional CFF a rather limited tool. Visual scientists consider it essential to determine CFF with a range of luminances from scotopic to photopic, and to vary the contrast (brightness difference) between the brightest and dimmest levels of the flickering stimulus. An arrangement devised by W. H. Merigan trains monkeys to perform on such a test. Fig. 2 plots a temporal visuogram from a monkey previously poisoned by methylmercury. This plot yields two conclusions. First, the sensitivity of this monkey's visual system to dim light levels is greatly impaired at all frequencies. Second, it also is impaired at high light levels when tested at high frequencies--an unexpected finding.

A similar modeling of frequency sensitivity can be performed spatially by modulating a target to display alternating lighter and darker segments. Fig. 3 demonstrates simultaneously the two parameters that can be examined. One is

spatial frequency, or the density of light and dark bands. The other is contrast, or the brightness difference between the light and dark bands. The brightest and dimmest areas represent the maximum and minimum values of a sine wave. In primates, maximum sensitivity is detected at about 10 cycles/degree of visual arc. A complete psychophysical mapping, as in temporal determinations, requires a range of spatial frequencies, a range of mean luminances, and a range of contrasts (modulation depths).

Performing these studies in monkeys is time-consuming and expensive. But what is the alternative when we insist on extrapolation to humans? Consider an issue currently in the news. In the next few years, we are bound to see large-scale development programs in methanol production for motor fuel. We know that advanced methanol poisoning causes irreversible deficits of the kind depicted in Fig. 4. What about the early stages? Do functional, reversible changes in vision serve as an early warning system? Does low-level, chronic exposure pose a danger from cumulative toxicity? Even if the chemical mechanisms of acute toxicity are worked out (Hayreh, in press), can the regulatory agencies justifiably extrapolate to chronic effects? Can anyone devise a way to assess subtle functional impairment without demanding such performance from the subject? That is what psychophysics does, in fact. And, if the subject is an animal, we teach it to signal us not by speech, but by some other response.

Let me sketch another example, again beginning with methylmercury. The current FDA guideline for fish originates in epidemiologic evidence that the earliest symptom of methylmercury poisoning is paresthesia, that is, numbness and tingling sensations. They are localized around the mouth and fingertips. Like other subjective phenomena, paresthesia waxes and wanes in an individual,

and whether a person reports it probably also depends on the cultural setting and who asks the question. A laboratory model for impairment of tactual sensitivity is just as important as one for vision, and for equivalent reasons. Paresthesia, however, given the speech limitations of most monkeys, is not a feasible choice. It also cannot be placed under experimental control; what would we use as the stimulus?

Our strategy was to employ vibration. It has several attractive features. One is that it is variable along the dimension of frequency, bringing it conceptually into the same scheme we apply to tests of vision and of hearing. We also know, from neurophysiology (Mountcastle, 1972), that vibration sensitivity is subserved by at least two receptor types, or, at least, two subsystems. One is maximally sensitive to low frequencies (about 30 Hz) and one to high frequencies (about 300 Hz). Vibration might be said to represent, not a primary sensory system, but a derived quality, which allows us to exercise the primary somesthetic processes subserving it.

The method developed at Rochester (Maurissen and Weiss, in press) is illustrated in Fig. 5. It serves in both human and animal studies. The subject's finger is maintained in contact with a vibrating rod whose frequency and amplitude of vibration are controlled by parameters specified in the computer program that controls the experiment. Both human and monkey subjects perform under a routine that requires them to report whether they have or have not detected a vibratory stimulus. Thresholds for human and monkey subjects yield functions that are almost identical. Fig. 6 shows the effects in monkeys both of methylmercury and of the drug misonidazole, a hypoxic cell radiosensitizer that can produce peripheral neuropathy in patients.

Like vision testing, this is a complex, heavily instrumented procedure. Once more, what is the alternative? The typical neurological tests for

somatosensory function are crude. Vibration is tested most often with a tuning fork whose amplitude is uncontrolled and whose single frequency may not be optimal for detecting deficits. Tactual sensitivity is so delicate that, for threshold determinations, vibration amplitude must be controlled to within 0.1 microns. Detection of early functional damage due to methylmercury, or to kepone, or to acrylamide, precludes reliance on gross clinical measures. Only precision instruments and tightly monitored and controlled procedures can tell us what is going on.

Aversiveness and Attractiveness

Psychophysics could be thought of as a discipline that quantifies subjective phenomena. An equivalent evaluation provides the basis for some of the decisions that regulatory agencies are pressed to make which may impinge only indirectly on issues of health. Is a vile smell toxic? Perhaps odors seem remote from environmental toxicology, but respiratory irritants are not. Do their aversive properties at low concentrations presage morphologic damage at high concentrations? The irritant properties of ozone, acroleins, peroxyacetyl nitrate, and other airborne contaminants, however, cannot be measured on a gauge. The organism must be asked. It is not sufficient simply to measure respiratory variables.

Humans can complete rating scales or assign a number to aversiveness. But animals? Some authorities believe that such subjective variables cannot be measured in animals or nonverbal humans. Such a claim contradicts decades of success by experimental psychology. Suppose we wished to measure the aversive potency of ammonia in mice. An appropriate model was designed in our laboratory by Wood (1979). The subject is studied in a Plexiglas chamber through which a stream of air constantly flows (Fig. 7). On specified

occasions, ammonia is added to the airstream. By poking its snout into one of the two cones attached to the walls of the chamber, the mouse interrupts a photocell beam, an event that is programmed to cut the flow of ammonia. Poking its snout into the other cylinder does not terminate ammonia. Mice learn to use the correct cylinder, and, as shown in Fig. 8, terminate higher concentrations of ammonia earlier and more frequently than lower concentrations. The mouse, in essence, tells us how much the ammonia bothers it. Such an assessment will be necessary for many new agents, and we cannot ascertain the relationships among aversiveness, respiratory impairment, and pathologic damage in humans.

The converse question is also critical. Is a volatile organic solvent a candidate for abuse as a recreational agent? This is another issue superficially distant from environmental toxicology, but it has now become a legitimate question. Should a chemical be cleared for household use if it exhibits the potential for wide abuse, particularly by children? TSCA does not deal specifically with this problem, nor does the Consumer Product Safety Commission, perhaps because it seems so difficult to evaluate. Yet, aggressive promoters are marketing agents such as butyl nitrite, mainly to young people, without interference.

Like aversiveness, "attractiveness" is amenable to an experimental analysis. Equipped with an intravenous catheter, and given access to a response device such as a lever or button whose activation can be programmed to deliver drugs through the catheter, animals will self-administer almost any of the drugs that humans abuse: morphine, heroin, cocaine, amphetamines, barbiturates, alcohol, nicotine. We reasoned that volatile agents also might appeal to monkeys, given that such agents are widely self-administered by humans. We then demonstrated that monkeys will push a button to produce a

flow of nitrous oxide, an agent highly regarded by dentists and Hollywood (Wood, Grubman and Weiss, 1977). Toluene, the volatile component of model glue, also supports self-administration by monkeys (Fig. 9). Suits by workers and their families pending against manufacturing plants where such substances were not adequately controlled, a situation promoting abuse and dependence, testify to the low probability that such problems will vanish if ignored.

Microwaves represent a physical hazard. No one disputes their capacity for damage at high power densities. After all, they cook meat. They provoke controversy mainly because adverse biological effects are claimed to stem from exposures too low to heat tissue. Soviet and Eastern European specialists are the most avid promoters of this thesis. To test these claims, American investigators, among other tactics, have attempted to measure tissue heating at low power densities by a variety of exotic methods. I use the term "exotic" because ordinary methods of temperature measurement are unsuitable. The sensing elements absorb heat themselves, so cannot report the temperature of the surrounding tissue. But suppose one could, in the intact animal, measure temperature reliably in many different body locations. Would even that reflect the integrated thermal burden?

We can adopt another strategy: employ the whole animal as a thermometer, so to speak. A long series of studies (for example, Weiss and Laties, 1961) had already established that rats and other animals, if given access to a device that operates a heat lamp or other heat source, will turn on the lamp at low ambient temperatures, and adjust this behavior in accord with the output of the source. Why not, we reasoned, determine how a rat responds for heat from a heat lamp when it is simultaneously being irradiated by a microwave source? In trained animals, we measured responding for infrared heat alternating 15-minute periods with and without simultaneous microwaves

(Stern et al., in press). Fig. 10 shows that a power density as low as 5 milliwatts/cm² significantly reduced responding for heat from the heat lamp. By asking the rat how warm it felt, we were able to demonstrate a clear thermal effect at the power density now serving as the U.S. exposure standard.

Developmental Disorders

Teratology testing is now an almost universal requirement in evaluating toxicity. The motives for such a requirement are visible in the debates that swirl about nearly every current environmental disaster: the Love Canal in New York State; the dioxin release at Seveso; Three-Mile Island. They derive from our recognition that the fetus and neonate lack many of the mechanisms enabling more mature organisms to withstand the biological impact of contamination. Although traditional teratology is built on morphology, the elevated susceptibility of the developing organism is reflected by functional as well as structural deficits.

Let me plagiarize an example first proposed by David Rall. Suppose that the ultimate consequences of the thalidomide disaster, rather than deformities among offspring of the treated mothers, had been a mean loss of 10 percent in I.Q. scores. Would we yet suspect thalidomide of adverse effects, even though such a loss represents about one standard deviation on the Stanford-Binet intelligence test? Such issues are the special province of Behavioral Teratology. Although a paradoxical fusion of terms, its significance is recognized officially. Both the Japanese and British governments now require behavioral assessments of the consequences of prenatal exposures as a part of toxicity testing. Sometimes, however, these consequences may not emerge until maturity or even advanced age. A massive experiment with methylmercury, for

example, revealed that exposure during gestation may portend deficits in mice that are not manifested until they reach the age of eighteen months, or about half their total life span (Spyker, 1975). We also know that psychotropic drugs given during gestation may leave functional scars that remain superficially invisible, but detectable by behavioral test methods.

Many different approaches to postnatal assessment are possible. Some rely on simple observation and reflex testing of neonates. But the neonate possesses such a limited repertoire of behaviors that only the grossest abnormalities can be discerned at that stage, and, in addition, the abnormalities are likely to be transient. Later testing, though more sensitive and significant, is likely also to be more complicated and expensive. If monkeys are treated from birth with enough lead to bring blood levels up to about 60 $\mu\text{g}\%$ at 200 days of age, they will show behavioral alterations at 2-3 years of age--at a time when blood levels have fallen to about 30 $\mu\text{g}\%$. Such levels are seen in many urban children. In the monkeys, rather refined behavioral test methods were able to reveal differences such as those based on the distribution of responding over time (Rice and Willes, 1979; Rice, Gilbert and Willes, in press).

These problems are amplified by the impact of advanced age on brain function. Consider Fig. 11, which depicts the "normal" decline of brain function (compiled by Kety, 1956) and the additional decrements that might be imposed by contaminants that act by destroying nerve cells or impairing their function (Weiss and Simon, 1975). Even though the influence of a process that accelerates the normal rate of aging by as little as 0.1 percent is undetectable in the short term, it makes a significant quantitative contribution over a fifty-year period. Figure 11, moreover, does not include the most vulnerable period of the life span-- fetal and neonatal life, when the brain and its supporting functions are incompletely developed.

The bewildering alternatives from which test approaches can be selected is generating pressures to mandate a behavioral teratology test battery. Several laboratories plan to compare interlaboratory reliability as the first step toward standardization. Even the brief discussion in this paper should help you appreciate the enormous scope and complexity of the issues. Reliability is a premature issue because we do not yet have a grasp of how to ask the critical questions--the issue of validity, or which procedures work.

Behavioral Epidemiology

So far, I have discussed tactics that permit us to extrapolate from laboratory animal models to humans. I have emphasized aspects of Behavioral Toxicology aimed at the more subtle questions posed by hazard assessment. As difficult as these tasks may prove, they are no match for the difficulties we encounter when studying human populations in their customary surroundings. With their staggering polymorphism and wildly divergent histories, human populations seem to tug us into investigative quicksands.

We study human populations, of course, because our ultimate criterion is their welfare and because extrapolations from the laboratory are imperfect predictors of human reactions. Even when the endpoint is comparatively unambiguous, as with cancer, epidemiology is an unruly enterprise. Consider what further insults Behavioral Epidemiology has to suffer: the victims don't appear on death certificates, a concrete diagnosis of a functional disturbance is almost self-contradictory, and many of the adverse effects are defined only by how the subjects feel.

The toxicology of metals, a Rochester specialty, offers many examples of these difficulties. Fig. 12 is a compilation of neurological and psychological symptoms linked to various metals. Don't quiz me too carefully

about my sources. They may represent obscure case studies, appealing epidemiology, and uninterpretable experiments. The figure is meant only to acquaint you with the bewildering scope of nervous system toxicity for which metals have been indicted and to stress the number of complaints that are predominantly subjective and/or nonspecific.

Mercury is one of the most fascinating examples. Most of you are aware that mercury vapor intoxication is marked by tremor. In fact, because mercury occupied an important niche in the processing of fur for felt hats, this neurological manifestation became known as "hatter's shakes". But even before tremor became apparent, the victims exhibited a cluster of psychological disorders consistent enough to earn a label: erethism, from the Greek for "irritation" or "red". The characteristic features of erethism are listed in Table 2. It is easy to see why clinicians may be misled by a patient who presents such a spectrum of complaints: they resemble what many of us experience at one time or another in our own lives, particularly around the time of a site visit.

Such nonspecific entities are more than a medical curiosity. They could constitute the main elements of policy decisions by regulatory agencies and even dominate the basis for legal action. The aftermath of the episode in the state of Michigan, during which thousands of dairy cattle were poisoned by polybrominated biphenyls inadvertently substituted for a feed supplement, is being debated on exactly such grounds. Farm families that consumed meat from the poisoned animals and their milk products claim to be suffering damage to their health as a result. What are their complaints? They resemble a recital of the symptoms of erethism (Table 3). What is the validity of such claims? The farmers, with a lawsuit pending, surely discuss the symptoms among themselves, and are naturally upset and depressed by the economic consequences

of the episode. Objective verification of the complaints seems no more than a remote possibility. These are epidemiological dilemmas that lurk in virtually every human exposure.

One of the most tenacious obstacles to epidemiologic deductions is the colossal range of susceptibility that marks the human population. It impedes our ability to predict allergenicity, a problem amplified by the lack of any suitable animal model. Questions of unique susceptibility (in contrast to graded pharmacological sensitivity) are relevant to all of environmental toxicology, but especially nag Behavioral Toxicology, because behavior almost never reflects only a single underlying mechanism. An arresting illustration of how the public's appreciation of this issue is changing the practices of food processors is the agitation about food additives and the syndrome called hyperkinesis. The origins of the controversy are almost completely the creation of a pediatric allergist from San Francisco (Feingold, 1973, 1975). On the basis of certain unexpected clinical findings, he began to pursue a possible relationship between elevated sensitivity to certain constituents of the diet and the behavioral disorder called hyperactivity or hyperkinesis. The constituents comprised synthetic colors, synthetic flavors, and what were termed "natural salicylates" in certain fruits and vegetables. He has promoted an elimination diet, that excises these constituents, as a form of therapy for hyperkinesis, and claims that 30 percent or more of afflicted children improve on such a diet.

Feingold's assertions have been attacked by elements in the food industry disturbed by the prospect of more detailed labeling regulations, and by various professionals, such as many pediatricians, stubbornly reluctant to believe that widely consumed diet constituents could play such a role without anyone uncovering it earlier. Arrayed against the industry and the professionals are parents groups calling themselves Feingold associations.

Although Feingold's claims were based on testimonial evidence and clinical observations, they stimulated several controlled clinical trials. Among them is one that I designed and that was conducted in California with a sample of children enrolled in the Kaiser HMO (Weiss et al., in press). We studied 22 children between about 3 and 7 years old. Each child had been maintained, with some improvement in behavior, on an elimination diet for at least 3 months before the study began. A child participated for 11 weeks. Every day, at a specified time, he or she drank a bottle of a specially-formulated soft drink. On 8 occasions, randomly dispersed among weeks 3-10, the drink contained a blend of 7 FD&C food colors in the quantities shown in Table 4. The control and challenge drinks were indistinguishable by sight, smell, and taste. Parent observations comprised our data source. For each child, the parent selected a set of 10 target behaviors, 7 aversive and 3 positive, from a repertoire of over 300 behavioral items drawn from a variety of inventories.

We found one mild and one spectacular responder to this rather modest and infrequent dose of colors. The latter was a 3-year old female. Fig. 13 charts her responses according to the Conners Parent-Teacher Questionnaire, a widely-used rating scale in hyperkinesis research. She also responded so consistently that her mother guessed she had been challenged 6 times, and was correct 5 times, giving a probability of correct guessing by chance of $p=1.6 \times 10^{-5}$.

A single striking case does not confirm Feingold's hypothesis. It is only strong evidence that the phenomenon of sensitivity exists. In the context of the other controlled trials, however, it takes on far more cogency, because the total data clearly confirm that at least some children respond to color challenge, and that the elimination diet reduces the severity of hyperkinetic behaviors in a subset of children. These supporting data came from two

controlled trials of the total diet and six color challenge experiments. Animal behavior studies also support the hypothesis. Neonatal rats treated with synthetic colors at dose levels equivalent to our California study display enhanced locomotor activity, and deficits in learning (Shaywitz et al., 1979). In vitro experiments also indicate a pharmacologic effect of artificial colors (Clafferman and Silbergeld, 1979). This mounting evidence emphasizes the necessity for Behavioral Toxicology in the testing of food additives, a point of view also held by the review panel that surveyed the GRAS list of the United States Food and Drug Administration (Sice et al., 1977).

Children are also the main targets in the other dominant themes of behavioral epidemiology. The consequences of asymptomatic but elevated lead body burdens probably have stirred the most vigorous debate. No one now questions the adverse potential of overt lead encephalopathy. But how does one establish a safe level from the premise that elevated lead body burdens, in the absence of clinical signs, can impair intellectual potential, academic performance, and social behavior? The literature is filled with valiant attempts to find an answer, but they have been hampered by the overwhelming problems of adequate control groups, the inadequacy of blood lead as an index of previous exposure, and small sample sizes. Because of these inadequacies, a recent study by Needleman et al. (1979) has come to dominate the issue. These investigators studied over two thousand school children in the Boston environs. They estimated lead exposure by collecting deciduous teeth, which give an integrated exposure index, and collected teacher ratings of academic performance and conduct. Consistent relationships surfaced between tooth lead concentration and teacher ratings, indicating that a threshold level for lead toxicity is a meaningless measure.

Conclusions

Behavioral questions are embedded in many facets of environmental toxicology. Despite the expense, the formidable capital investment required, and the often frustratingly slow pace of behavioral investigations, we still have no substitute for the whole organism as an assay system.

One of the most convincing arguments for Behavioral Toxicology was made, not by one of us current upstarts, but by F.H. Lewey in his classic article (1941) on carbon disulfide poisoning in the viscose rayon industry:

"The majority of signs of disease here mentioned were discovered years ago without the aid of modern apparatus. There is no doubt that a piece of cotton wool and a pin are all that are necessary to detect motor weakness, sensory loss, decrease of corneal and pupillary reflexes, provided that the disturbance is massive enough to be seen with the naked eye. However, CS₂ intoxication is a good example of the experience that reliance on the "established" qualitative methods has led to erroneous statements such as that of the frequent occurrence of poor vision and hearing and, on the other hand, the overlooking of early stages of peripheral neuropathy and of the beginning decrease of corneal and pupillary reflexes. We like to stress the point that to our knowledge there is no way of early detection of this and many other neural diseases except by the application of the most sensitive apparatus giving numerical and reproducible results. The time has passed when the physician could complacently wait until his patients' symptoms and signs had reached the point where diagnosis could not possibly be missed. The recovery period from CS₂ poisoning is closely related to the hours of exposure. No

instrument should be considered too complicated if with its help the time of absenteeism could be shortened. We have learned, finally, that in dealing with a great number of persons suffering from the same disease, and in integrating and combining their symptoms and signs, figures cannot always be taken at their face value but have to be examined for their significance. It should be understood that statistics is not a method of twisting the facts but of testing the validity of their interpretation."

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Figure Legends

Fig. 1. Effects of a 29-week exposure to methylmercury on visual discrimination performance of treated monkey (macaca arctoides). The animal was trained to select a square stimulus projected on one of three response keys. The other keys contained a circle or a triangle. Squares denote performance when selecting one of the three forms. Triangles denote performance when only the square was projected (a brightness discrimination). Methylmercury blood concentration was maintained at about 3 ppm.

Fig. 2. Flicker sensitivity of a methylmercury-poisoned monkey. The dashed line represents performance by a control monkey; that is, the monkey's ability to detect flicker at various frequencies of flicker. Detection sensitivity corresponds to modulation depth, or the difference in brightness between the brightest and dimmest portions of the flicker cycle. The poisoned monkey's performance is worst at low luminance levels (scotopic or night vision). Even at medium and high luminance levels, however, this monkey is impaired, because it cannot resolve high frequency flicker. This is a permanent loss because the monkey was poisoned three years earlier.

Stimuli were presented via two oscilloscopes. The monkey responded by pushing a key corresponding to one of the oscilloscopes. Responses to the correct (flickering) oscilloscope were followed by presentation of a small quantity of fruit juice through a spout.

Fig. 3. Grating patterns employed in developing a spatial visuogram. Spatial frequency is represented as the density of light and dark bars, with the number of alternating bars subtending one degree of visual arc equal to frequency. The inset shows the luminance profile of one cycle. The pattern on the left is lower in spatial frequency and higher in contrast than the one on the right. (From Bodis-Wollner, I., and Diamond, S.P. The measurement of spatial contrast sensitivity in cases of blurred vision associated with cerebral lesion. Brain 99: 695-710, 1976.

Fig. 4. Pattern of visual loss produced by methanol. In contrast to methylmercury, which damages the peripheral areas of the visual field, methanol impairs the central field. Parameters of functional loss correspond to sites of morphologic damage.

Fig. 5. Vibration testing apparatus. For monkey testing, the pair was stabilized in a plasticene cast.

Fig. 6. Vibratory thresholds in two monkeys. M-802 was treated with the hypoxic cell sensitizer misonidazole. M-800 was treated with methylmercury. Open circles represent thresholds before treatment, closed circle thresholds after treatment. Dashed and solid lines represent quadratic curve fits before and after treatment respectively. Vertical bars denote one standard error of the mean; each point is based on 10 determinations.

Fig. 7. Schematic of ammonia escape technique. By interrupting the photocell beam, the mouse terminates ammonia flow.

Fig. 8. Average duration of ammonia delivery as a function of ammonia concentration. These data represent the mean performance of six mice given one session of 50 deliveries at each concentration, except for the 0.1% level, which is the mean of the means of 4-10 sessions per mouse. Plotted are the means ± 1 S.D.

Fig. 9. Self-administration of toluene by a squirrel monkey (Saimiri sciureas). Each button-pushing response by the monkey produced a 15-second flow of toluene when the flow was inactive. The designations 0, I, and II represent control conditions. Each + denotes a separate 1-hour experimental session.

Fig. 10. Behavioral thermoregulation in six rats as modified by microwave exposure. Rats whose fur was dipped pressed a lever to turn on an infrared heat lamp. At an ambient temperature of 20°C, while responding steadily for 2-second bursts of heat, a microwave source was turned on for alternating 15-minute exposures at power densities of 5, 10, or 20 milliwatts/cm². Simultaneous microwave exposure reduced responding for infrared heat. The function, based on the mean of six rats, demonstrates that the amount of time that the infrared lamp was kept on during microwave exposure periods was less than during the directly preceding control period.

Fig. 11 A (Top). Functional capacity of the human brain, in terms of neuronal cell density, plotted against age. The heavy line is based on Kety (1956). The lighter lines represent extrapolations from excess rates of cell loss (Weiss and Simon, 1975).

B (Bottom). Transformation of the plots in 10A to show chronological age versus equivalent brain age for different rates of excess brain aging. Zero percent is equal to the heavy line in 10A and represents no deviation from "normal" aging.

Fig. 12. Matrix showing clinical complaints associated with alleged metal toxicity.

Fig. 13. Daily behavior ratings (by mother) of a target behavior ("Throws and breaks things") during a 77-day experimental period. The subject, a 34-month old girl, consumed an experimental soft drink each day. On days denoted by asterisks (*), the drink contained a blend of seven certified food colors rather than the caramel coloring and cranberry powder added to the control drinks. Lines connect color challenge days. A rating of 9 corresponded to extremely marked responses; a rating of 1 to only a slight response. A rating of 0 denotes no occurrence of that behavior on that particular day. Challenge days were significantly different from control days ($p < .0003$).

TABLE 1

INDICES OF METHYLMERCURY TOXICITY

- SENSORY
 - Paresthesia
 - Pain in limbs
 - Visual disturbances (Constriction)
 - Hearing disturbances
 - Astereognosis
- MOTOR
 - Disturbances of gait
 - Weakness, unsteadiness of legs; falling
 - Thick, slurred speech (Dysarthria)
 - Tremor
- OTHER
 - Headaches
 - Rashes
 - "Mental disturbance"

TABLE 2

SYMPTOMS OF ERETHISM

- Hyperirritability
- Blushes easily
- Labile temperament
- Avoids friends and public places
- Timid, shy
- Depressed, despondent
- Insomnia, fatigue

Table 3

Symptoms Associated With PBB Exposure
(Valciunas et al, 1978)

Depression

Nervousness

Tiredness

Paresthesia

Somnolence

Headaches

Dizziness

Vomiting

Loss of Balance

Dry Skin

Blurred Vision

Muscle Weakness

Nail Changes

Increased Perspiration

Difficulty Walking

TABLE 4

<u>COLOR</u>	<u>mg/DAY</u>	<u>S.D.</u>
Yellow 5	9.07	6.88
Yellow 6	10.70	8.95
Red 40	13.80	12.12
Red 3	0.57	.504
Blue 1	0.80	.706
Blue 2	0.15	.127
Green 3	0.11	
TOTAL	35.26 mg.	
RANGE	.382 - 184.80 mg.

FIG 1

FIG 2

FIG 3

METHANOL

FIG 5

FIG 6

FIG 7

FIG 8

FIG 9

FIG 10

FIG 11

	ALUMINUM	ANTIMONY	ARSENIC	BORON	CADMIUM	LEAD	MANGANESE	MERCURY	NICKEL	SELENIUM	TELLURIUM	THALLIUM	TIN	VANADIUM
ANOSMIA			•		•									
APPETITE LOSS		•						•			•			•
CONVULSIONS						•								
DEPRESSION				•						•				•
DISORIENTATION				•								•		
DIZZINESS		•	•						•	•		•	•	
DYSARTHRIA	•						•	•						
FATIGUE, LETHARGY		•	•		•			•		•				
HEADACHE		•	•	•			•	•	•				•	•
INCOORDINATION, ATAXIA	•					•	•	•				•		
INSOMNIA									•					
JITTERINESS, IRRITABILITY		•		•					•					•
MENTAL RETARDATION						•		•						
PARALYSIS	•													
PARESTHESIAS		•	•					•					•	
PERIPHERAL NEUROPATHY			•			•		•						
POLYNEURITIS		•	•										•	
PSYCHIATRIC SIGNS	•					•	•	•	•					•
SOMNOLENCE											•	•		
TREMOR	•					•	•	•						•
VISUAL DISTURBANCES						•	•	•		•			•	•
WEAKNESS			•	•		•	•	•	•	•				•

SYMPTOMS ASCRIBED TO METAL TOXICITY

FIG 13