Empirical Approaches for the Investigation of Toxicant-induced Loss of Tolerance

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It has been hypothesized that sensitivity to low-level chemical exposures develops in two steps: initiation by an acute or chronic chemical exposure, followed by triggering of symptoms by low levels of previously tolerated chemical inhalants, foods, or drugs. The Working Group on Toxicant-induced Loss of Tolerance has formulated a series of research questions to test this hypothesis: Do some individuals experience sensitivity to chemicals at levels of exposure unexplained by classical toxicological thresholds and dose–response relationships, and outside normally expected variation in the population? Do chemically sensitive subjects exhibit masking that may interfere with the reproducibility of their responses to chemical challenges? Does chemical sensitivity develop because of acute, intermittent, or continuous exposure to certain substances? If so, what substances are most likely to initiate this process? An experimental approach for testing directly the relationship between patients' reported symptoms and specific exposures was outlined in response to the first question, which was felt to be a key question. Double-blind, placebo-controlled challenges performed in an environmentally controlled hospital facility (environmental medical unit) coupled with rigorous documentation of both objective and subjective responses are necessary to answer this question and to help elucidate the nature and origins of chemical sensitivity. — Environ Health Perspect 105(Suppl 2):515–519 (1997)

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Introduction and Definitions

It has been hypothesized that sensitivity to low-level chemical exposures develops in two steps: induction or initiation by a chemical exposure event, followed by triggering of symptoms by low levels of the same substance or chemically dissimilar substances (1–5). Possible initiators include single, high-level exposures or chronic (intermittent or continuous) lower level exposures. Triggers include common

inhalants such as fragrances, traffic exhaust, foods, drugs, alcohol, and caffeine. Although anecdotal clinical evidence supports this hypothesis, carefully conducted epidemiologic and experimental studies are needed for its corroboration or rejection.

A series of research questions designed to test the two-step mechanism proposed above was devised. While other working groups focused on conditioning and learning, psychoneuroimmunology, neurogenic inflammation, and neural sensitization as possible explanations for chemical sensitivity, the Working Group on Toxicant-induced Loss of Tolerance developed an approach to test directly the relationship between specific exposures and patients' reported symptoms and physiological responses.

Double-blind, placebo-controlled human challenge studies are essential to understand the nature of chemical sensitivity. Research focusing on exploring particular mechanisms while excluding others at this early stage of scientific investigation could lead to blind alleys. Pivotal questions about the origins of the condition, such as the role of chemical exposures compared to that of individual perception, may remain unanswered. In the tradition of scientific inquiry, research in this area should proceed from the general to the specific. Once the general nature of chemical sensitivity is understood (e.g., toxigenic, psychogenic), particular mechanisms can be explored. Scarce resources must not be expended on intriguing but irrelevant hypotheses.

Defining whether chemical sensitivity is physiological in origin or psychogenic is important for many reasons, including policy setting, prevention, and selection of appropriate therapies. However, investigators in this area must be cautious in interpreting their experimental observations because subjects' responses to challenges may involve both physiological and psychological elements. For example, psychological symptoms such as depression or irritability may be psychogenic or organically based, or both. Subjects who fail to respond to active challenges may respond differently under other circumstances. Blinding odorous substances is a task subject to numerous pitfalls.

Several terms were defined. Loss of tolerance is defined as the loss of natural or native tolerance for common substances such as fragrances, traffic exhaust, foods, caffeine, or alcoholic beverages. The term refers to the reported finding that some

persons exhibit adverse reactions to chemicals that they formerly did not exhibit and that others do not exhibit at the doses or levels administered. This definition is in contrast to acquired tolerance, e.g., to a drug, which is defined as reduced effect as a function of dosage. Masking, discussed in detail by Miller (6), refers to the hiding or obfuscation of responses to challenges due to overlapping of responses to closely timed exposures, or habituation or acclimatization to incitants. Triggering is defined as the provocation of symptoms by a chemical stimulus or incitant.

Ultimately it will be important to answer the question whether exposure to pesticides, contaminated air in sick buildings, or low levels of solvents can initiate chemical sensitivity. From a public health and prevention point of view, this question is paramount.

It may seem logical first to address the initiation stage in the development of chemical sensitivity. Practically speaking, however, triggering should be addressed first because if challenge studies on triggering show that most symptoms are psychogenic in nature and not reproducible, there is less need to study the initiation phase. The working group suggested three principal questions for study. The first two relate to triggering, whereas the final question explores initiation.

1) Do some individuals experience sensitivity to chemicals at levels of exposure unexplained by classical toxicological thresholds and dose–response relationships, and outside normally expected variation in the population? (Does triggering occur?)

2) Do chemically sensitive subjects exhibit masking that may interfere with the reproducibility of their responses to chemical challenges? (Does masking affect responses?)

3) Does chemical sensitivity develop as a consequence of acute, intermittent, or continuous exposure to certain substances? If so, what substances are most likely to initiate this process? (What initiates the condition?)

The first question, related to triggering of symptoms in some individuals, is perhaps the most fundamental and received the most attention.

Several experimental considerations were raised. No attempt was made to develop a single rigid experimental design. It was recognized that in the first round of studies that selection of dependent variables may need to be open-ended because

of the diversity of clinical presentations and reported incitants. For example, pulmonary function testing would be appropriate for patients who reported shortness of breath but not for those whose primary symptoms were headaches or depression. A survey of patients' responses to various exposures could help identify those variables most likely to be responsive under challenge conditions. In addition, selection of exposure agents and concentrations may need to be tailored to each subject during pilot studies. Following the first round of studies, a series of studies will be needed to focus on selected chemicals and symptoms.

General Approach and Methodology

During the pilot studies, subjects would be housed in an environmentally controlled medical unit (EMU) (1,7,8). Following an estimated 4- to 7-day period of avoiding chemical and food incitants (unmasking), subjects would be challenged with a selected number of chemicals and foods in a double-blind, placebo-controlled manner. The 4- to 7-day window of time estimated as necessary for unmasking is empirically derived from clinical observations by chemically sensitive patients and some of their physicians. Masking itself requires testing. A rationale for the 4- to 7-day window is discussed elsewhere in this issue by Miller (6). Four to seven days is the average length of time reported for patients' symptoms to resolve after they have begun to avoid incitants. Although the term detoxification has been used to describe this process, it has a connotation that differs from unmasking. For example, for persons dependent on alcohol, detoxification (sudden cessation of alcohol consumption) might result in elimination of alcohol from their systems in a day or more. On the other hand, it might be several days before receptors normalized and full recovery occurred (unmasking).

Testing of the masking concept is addressed more fully in the following discussion. Important empirical questions remain to be answered concerning masking, questions that may be crucial to the timing of exposure challenges and to the outcome of those challenges.

Subject Selection

Subjects should be individuals sharing recent similar initiating exposures; for example, persons who became ill during the remodeling of a building or who were exposed to organophosphate pesticides

similar to Miller and Mitzel's remodeling- and organophosphate-exposed groups (9). Persons with chronic diseases or with sensitivities that developed gradually over their lifetimes would not be good subjects for pilot studies because the greater uncertainties about the origins of their illness would further complicate scientific inquiry. Subjects who report disruptions in career and lifestyle by their symptoms and intolerances would be preferable for such a study so as to increase the likelihood of robust responses with challenges. An alternative approach might be to study groups of individuals representing particular stages in the evolution of the condition, perhaps evidenced by different symptom severities and time elapsed since initiating events.

Subjects 18 to 55 years of age are recommended. Self-reported symptoms are not as reliable for children, particularly those 12 years of age or younger, and after age 55, end-organ disease and the ability to endure the rigors of chemical challenges are of concern. Equal numbers of symptomatic male and female subjects might be chosen. Alternatively, selection without regard to gender (gender-blind recruitment) could be implemented. Based upon clinical experience to date, approximately 80% of subjects might be expected to be women in the latter case.

Individuals requiring or dependent upon tobacco, alcohol, or prescription or nonprescription drugs should be excluded as candidates because these substances could alter responses to challenges. It would be important for all participants to be at a "clean baseline" with respect to chemicals, foods, and medications before any chemical challenges.

Other reasons for excluding subjects from study include diagnoses of borderline personality disorder, schizophrenia, or other psychiatric disorders that might interfere with sustained cooperation or the ability to implement lifestyle changes should these be found necessary. Structured clinical interviews and instruments for personality assessment will help ensure the consistency of these determinations. Patients with mild to moderate depression could participate provided they have no history of suicide attempts or threats, they are not taking antidepressants, and standard suicide precautions are implemented. It has been reported that after withdrawal from chemical, food, and drug incitants, and following some challenges, depression may increase transiently (7).

There were differing opinions about including subjects involved in litigation

or compensation proceedings. Concerns about the possible impact of litigation upon subjects' responses further underscore the need for double-blind, placebo-controlled challenges.

Control subjects could be selected in various ways—for example, healthy, normal individuals; anosmics; or patients with orthopedic injuries. Matching for age, gender, and education is advisable. Control subjects can help define the expected responses of normal individuals in the unique environment of an EMU regarding withdrawal, masking, and other parameters. In addition to the use of control subjects, subjects could act as their own controls, for example, in a repeated measures experimental design. It is recommended that a repeated measures design be used both with patients serving as their own controls and those serving as normal controls (persons not known to be sensitive to chemicals) to define the impact of the EMU and the protocol on both putatively sensitive and normal individuals.

Experimental Design

The sequence of procedures in a proposed experiment would be as follows: recruit subjects and controls; screen both subjects and controls; make baseline measurements; conduct open chemical and food challenges; conduct blind chemical and food challenges and measure dependent variables; debrief and reacclimatize participants.

Measurements made on subjects while still at home before they entered the EMU might include: volatile organic chemicals in breath or indoor air in subjects' homes; self-reported ratings of symptoms using visual analog scales; an inventory of life stressors; additional demographic data including level of education and socioeconomic status (important for selection of controls); detailed psychosocial, family, occupational, and environmental histories; and computerized neurobehavioral batteries of tests to measure cognition and attention span at baseline. Selected measures should be repeated following entry into the EMU, after unmasking, and before and after challenges.

For all subjects, the EMU experience would begin with a period of controlled exposure. To assure that subjects were at a clean baseline (unmasked) before testing, they would be housed in a hospital research area or a wing specially designed to reduce exposure to volatile organic chemicals, an EMU (1,7,8). The goal would be to reduce participants' exposures to airborne

contaminants to the lowest levels practicable. Fragrances, pesticides, disinfectants, soft plastics, and other chemicals and materials that emit volatile compounds would not be allowed in such a facility. Clean air for breathing would be achieved through the use of materials and furnishings that do not emit volatile compounds, state-of-the-art air filtration for particulates and vapors, and controlled access to the unit.

The rationale here is that a hospital-based environmental medical unit would permit residence for a time sufficient to allow patients to unmask fully and reach a clean (asymptomatic or minimally symptomatic) baseline. Round-the-clock nursing and hospital emergency services necessary to ensure patient safety generally are not available in conventional exposure chambers. During unmasking and subsequent challenges, chemically sensitive patients have been reported to develop bronchoconstriction, confusion, depression, and other symptoms that mandate they be under continuous observation (8).

Testing at admission to the EMU should include computer-administered cognitive batteries and subjective symptom ratings on a visual analog scale. Neurobehavioral tests should address subjects' attention, concentration, memory (verbal and visual), and general processing efficiency. Computer administration of performance tasks with prior training to asymptote in order to avoid practice effects could be performed during the unmasking period. In addition, odor thresholds, smell identification ability (UPSIT) (10), and odor intensity ratings could be evaluated. Odor thresholds should be determined using up/down staircasing.

Because it is not feasible to test every subject with every potential incitant, challenges could be administered using a defined panel of substances at entry into the EMU. This first round of testing should be conducted using an open challenge format to ensure that substances and concentrations selected for later blinded challenges are those reported to trigger symptoms. Similarly, open challenges with placebos should be performed before administering blinded challenges. This will help to ensure that for each subject, actives are truly actives and placebos truly placebos, since one person's placebo might be another's trigger and vice versa. All subsequent challenges should be double-blind and placebo-controlled. Symptoms and signs are then recorded and observed for resolution over time.

Following the 4- to 7-day period of unmasking, blinded challenges should be conducted using the same incitants used at admission. This approach to testing subjects—both on entry into the EMU and after unmasking—could help advance understanding of the possible effects of masking upon responses. Ideally there should be no contact between subjects before or during testing; however, this would be very difficult to achieve. The wisest option might be to house subjects together but test them separately.

Statistical considerations are important in determining the number of repeat challenges per incitant. Also, there are practical limits as to the number of challenges patients can be expected to undergo, particularly if their symptoms are severe. Careful consideration should be given to reacclimatizing subjects to real-world conditions on completion of their evaluation and before discharge from the EMU. A protocol might be designed for this purpose that consists of gradual reintroduction of less problematic triggers.

Even among chemically sensitive persons exhibiting highly individualized symptoms in response to many different incitants, it may be possible to study and compare subjects and controls in an informative way. Before subjects enter the EMU, baseline challenges could be conducted to characterize each subject's incitants and symptoms. Incitants such as formaldehyde or ethanol that are common to several groups of subjects may help define subgroups, as may particular symptoms such as breathing difficulties or gastrointestinal effects.

During the double-blind, placebo-controlled challenges, patients could be asked to report the severity of their symptoms on a scale (e.g., a 10-point scale) that would facilitate comparison of responses between or among different subjects and controls. For example, a subject might report symptoms of varying severity evoked by five alternating, sequential administrations of incitants and placebos to yield the readings: 6/3, 5/2, 7/4, 5/2, and 3/1. Subgroups exhibiting identical symptoms evoked by different incitants or affected by the same incitant but having different symptoms might be examined in this manner, as well as persons having different symptoms in response to different incitants. In each of these situations, with the subjects acting as their own controls during administration of the double-blind, placebo-controlled challenges, it would be useful to compare

the ratios of the responses to incitants over (divided by) the responses to placebos in the experimental subjects to the corresponding response ratios in the control group. For example, a control subject might exhibit the ratios 2/2, 3/2, 3/3, 1/1, and 2/1 to the five sequential, alternate administrations of incitants and placebos. The distribution of the ratios for the experimental subjects could be compared graphically to the distribution of the ratios for the control group and the response differences between experimental and control groups analyzed statistically.

Exposure Variables

Dose Level

Measures of exposure dose could include dose delivered, breath or blood concentrations, and/or amounts appearing in the urine.

Type of Exposure Administration

Double-blind, placebo-controlled challenges should be administered, with a 4- to 7-day interval between challenges of a particular type. While administration of ascending concentrations of challenge substances has advantages in terms of patient safety and ensuring delivery of an effective dose, such an approach could lead to short-term tolerance (as occurs in drug desensitization protocols) when, in fact, a single dose on the high side of the ascent might have provoked symptoms. The consequences of administering various concentrations at various intervals should be explored further.

Nasal Occlusion

The effects of occluding the nares (using a nasal clip or Microfoam TM tape [3M Corporation, St. Paul, MN]) during challenge compared with no occlusion should be assessed, given one hypothesis that olfactory–limbic stimulation plays a role in triggering symptoms of chemical sensitivity (5).

Specific Challenge Agents

A number of specific challenge agents were proposed, including inhalants such as vanillin/vanilla (no trigeminal stimulation) and carbon dioxide (trigeminal stimulation); fragrances such as galaxolide (musk), rose oil and geraniol; low concentrations of common solvents and indoor air contaminants such as ethanol, xylenes, decane, undecane, toluene, trimethyl benzenes, ethyl toluene, ethyl benzene, paradichlorobenzene (mothballs), methyl ethyl ketone, and acetone; possible controls such as

water, propylene glycol, and light mineral oil (the appropriateness of each of these as placebos would be confirmed via open challenge for each subject); ingestants administered in the form of capsules or as frozen slurries with nasal occlusion for blinding taste; caffeine; monosodium glutamate; various food additives; particular foods or sugars that evoked symptoms during open challenges; possible controls such as matched slurries or capsules using foods not associated with symptoms during open challenge—for example, beef as a control for pork or oatmeal as a control for corn.

Substances known to bioaccumulate such as polychlorinated biphenyls and chlordane should not be used for testing. The ethics of using certain common household pesticides even at very low concentrations as challenge substances was discuessed. Some reports suggest that certain pesticides might initiate chemical sensitivity in susceptible persons (4,9). Another consideration is the length of studies and cost involved in waiting 4 to 7 days between challenges of a particular type. Chemically distinct challenges could be administered in the intervals between challenges of a given type provided subjects had recovered fully before each challenge.

Dependent Variables

Dependent variables discussed for possible use include: duration of response; symptoms such as headache, fatigue, myalgias, and other symptoms rated on visual analog scales to record severity; ratings on mood scales such as the Profile of Mood States (POMS); performance, which includes such things as computer-administered cognitive batteries (with emphasis on attention span, memory, and concentration measures) and balance (posturography); physiological measures which include nasal resistance, pulmonary function testing such as peak flow readings and FEV_1, blood pressure, pulse, electrocardiogram, and galvanic skin response; and rheumatological indices such as rings for measuring finger joint swelling and dolorimeter readings for measuring pain.

Brief batteries for specific tasks could be alternated with continuous performance tasks. The duration of any testing sequence should not exceed about 20 min to be useful for administration before and after challenges.

As a practical matter, initial studies should focus on documenting signs and symptoms in response to blinded challenges, rather than costly shot-in-the-dark

attempts to find blood markers or perform brain imaging.

Ethical Considerations Involving Human Subjects

Subjects should be counseled about the initial withdrawal or unmasking phase of their evaluation and challenge testing during which symptoms temporarily may increase in severity. On-site emergency response capabilities and limitations should be explained to subjects. If they are found to be sensitive to particular chemicals or foods during the study, a transitional "safety net" for reentry into the "real world" should be incorporated into the protocol. Upon completion of the study, participants should receive any data pertinent to their well-being, including the results of all challenges, how to avoid substances to which they reacted, and how to use air filters and/or respirators if indicated. As with all research involving human subjects, informed consent is required. The consent should fulfill social responsibility yet avoid biasing subjects' responses.

The masking hypothesis, the second question suggested for investigation, could be tested using an approach similar to that discussed in question 1. In this case, however, subjects in the EMU would be challenged with incitants already known to provoke significant effects. Changes in the intensity and duration of symptoms with sequential challenges administered at varying intervals—for example, 10 days apart, 7 days, 5 days, 3 days, 2 days, and 1 day— would be monitored. Questions to be answered include: At what point does remasking occur? How far apart should challenges be spaced to observe the most robust effect following reexposure? What are the effects of lengthening or shortening the time interval between challenges of a particular type? Answers to these questions will facilitate the design of future studies.

The third question to be answered concerns whether chemical sensitivity develops as a consequence of acute, intermittent, or continuous exposure to certain substances. If so, which substances are most likely to initiate this process? If the research described above uncovered individuals exhibiting biological sensitivity at levels unexplained by classical toxicology, then research into particular mechanisms would be indicated. An additional task would be to determine whether and which exposures initiate chemical sensitivity.

Two basic approaches for examining question 3 were identified. One approach

would be to begin with a particular exposure history and try to determine whether some of those who had been exposed developed chemical sensitivities, and follow those who developed chemical sensitivities prospectively. Examples of selecting on the basis of exposure history might include identifying workers or community members exposed during a chemical spill or accident; persons residing in a building or community during pesticide application; workers or teachers and school children exposed in a sick building; patients receiving a particular anesthetic, implanted device, or other medical intervention; or consumers who purchased a particular product, such as new carpeting.

A second approach to question 3 would be to choose subjects with particular medical conditions, diseases, or symptoms and look for patterns of prior exposure. Populations of patients with Parkinson's disease, chronic fatigue syndrome, fibromyalgia, asthma, multiple sclerosis, peripheral neuropathies, chronic urticaria, chronic

sinusitis, and other conditions might be queried for chemical sensitivities and any history of an antecedent chemical exposure event. Recent work on Parkinson's disease suggests that living in a rural area and drinking well water may be risk factors for this condition (*11*).

The first approach, that is, tracking individuals with similar exposure histories to discover whether any subsequently develop low-level chemical intolerances, may prove more fruitful because these study groups would be relatively homogeneous.

Summary

Understanding toxicant-induced loss of tolerance or heightened responsiveness to low-level chemical exposures requires investigating both the triggering of symptoms and the initiation of chemical sensitivity in affected persons. Because of the wide variety of symptoms attributed to chemical sensitivity and the fact that chemical sensitivity could represent a general class or family of disorders, traditional

population-based epidemiologic approaches are unlikely to be helpful in documenting the triggering stages. Instead, individual double-blind, placebo-controlled challenges in which subjects act as their own controls are needed. A control group should also be investigated in parallel fashion and is particularly important for defining the effects of the EMU on responses. Because of its possible critical role, masking must also be investigated.

On the other hand, traditional population-based studies may be useful in studying the initiation of chemical sensitivity. Such studies are most likely to be successful when as many confounding variables as possible are minimized. A particularly useful strategy may be to select persons for study who shared the same or similar exposures preceding onset of their illnesses. The induction of chemical sensitivity is addressed in this paper as a third but equally important research direction, particularly in the context of preventing new cases of chemical sensitivity.

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