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 not previously experienced? 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 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., toxicogenic, 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 inhalants such as fragrances, traffic exhaust, foods, drugs, alcohol, and caffeine. Although anecdotes 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.

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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 these should 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 bronchocstriction, 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 placebo, 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 corre-
sponding 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 exper-
imental subjects could be compared graphi-
cally to the distribution of the ratios for
the control group and the response differ-
ences between experimental and control
groups analyzed statistically.

Exposure Variables

Dose Level

Measures of exposure dose could include
dose delivered, breath or blood concen-
trations, 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 toler-
ance (as occurs in drug desensitization pro-
tocols) when, in fact, a single dose on the
high side of the ascent might have provoked
symptoms. The consequences of adminis-
tering 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 chal-
lenge compared with no occlusion should be
assessed, given one hypothesis that olfac-
tory-limbic stimulation plays a role in trig-
gravity of symptoms from 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 stimula-
tion); fragrances such as galaxolide (musk),
rose oil and geraniol; low concentrations of
common solvents and indoor air contami-
nants such as ethanol, xylenes, decane,
undecane, toluene, trimethyl benzenes,
ethyl toluene, ethyl benzene, paradichloro-
benzene (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 glu-
tamate; various food additives; particular
foods or sugars that evoked symptoms dur-
ing 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
chlorodane should not be used for testing.
The ethics of using certain common house-
hold pesticides even at very low concentra-
tions as challenge substances was discussed.
Some reports suggest that certain pesticides
might initiate chemical sensitivity in sus-
ceptible persons (4,9). Another consid-
eration 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; symp-
toms 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 cog-
nitive batteries (with emphasis on attention
span, memory, and concentration mea-
sures) and balance (posturography); physi-
ological measures which include nasal
resistance, pulmonary function testing such
as peak flow readings and FEV1, blood
pressure, pulse, electrocardiogram, and gal-
vanic 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 perfor-
mance 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 chal-
lenges, 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 ini-
tial 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 com-
pletion of the study, participants should
receive any data pertinent to their well-
being, including the results of all chal-
lenges, 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, how-
ever, subjects in the EMU would be chal-
lenged with incitants already known to
provoking 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 con-
cerns 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.

REFERENCES