

Breathtaking Research

Dangers in the Air We Breathe

Although the air we breathe is necessary for life, certain factors may make this same air detrimental to our health. For instance, a seemingly endless list of compounds can be toxic at certain concentrations, and inhaling such compounds may lead to damage in different parts of the body. To investigate the particular effects of inhaled toxicants, the NIEHS developed the Respiratory Toxicology Group. The group, which consists of toxicologist Dan Morgan, biologist Cassandra Shines, and engineer Michael Moorman, has been conducting experiments for about a decade at the NIEHS inhalation facility. Morgan says the group typically conducts studies of chemicals that have been nominated to the National Toxicology Program for investigation into their carcinogenic potential and other end points. “We provide research support and conduct special studies on these chemicals,” he says. “We also do collaborative studies with other NIEHS investigators, other [federal] agencies such as the Environmental Protection Agency and the Department of Energy, universities, and industry.”

Nosing Around

The group uses two methods to perform inhalation experiments. In so-called nose-only exposures, the investigators place a rat

or mouse in a plastic device that looks like a soft drink bottle without a bottom, and a prescribed mixture of air is delivered to the animal’s nose through the neck of the “bottle.” In whole-body exposure studies, many animals—each in its own cage—can be exposed to toxicants simultaneously in a 1,000- to 2,000-liter chamber. In this technique, the investigators must make sure that the air mixture is the same throughout the chamber. Says Moorman, “When we set it up, we sample from all the corners and down the middle—18 different spots, typically—and verify that [atmospheric concentrations are] within 5%. When we actually run the chamber, samples are taken from one spot that’s representative.”

To get the proper mixture of air to the animals, the investigators use what Moorman calls “a couple of pieces of plumbing.” If they need to vaporize a liquid, one device sprays it into a glass pipe, where it evaporates into the air being delivered to the animals. If a compound is highly volatile or will be deployed at a very low concentration, vapor is drawn from the headspace of a vial containing the exposure compound. Metering pumps introduce the vapor to the airstream. All of these processes are monitored and controlled by a computer. What makes these studies tricky, Moorman says, is that they require documenting what happens inside the chambers—“guaranteeing that the atmospheric concentration is what we plan for it to be and accumulating the data to support that,

which includes temperature, humidity, and the time of day that we do things.”

The investigators use different devices to measure the amounts of different gases being inhaled by the test animals. With mercury, for instance, they rely on a dedicated analyzer. To measure the amounts of most common gas vapors, they use a gas chromatograph with a photo-ionization detector. During an experiment, the investigators might also gather physiological and biochemical data through sampling of blood or urine, measuring changes in body temperature, or determining respiratory rates of the test animals while they breathe the exposure atmosphere.

Of Mice and Men and Mercury

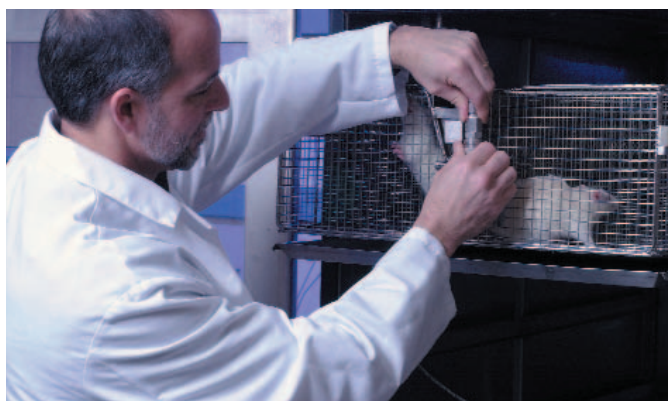
Currently, the Respiratory Toxicology Group is exploring the health effects of inhaling elemental mercury vapor. Says Morgan, “The first mercury study we did was in response to studies conducted by NIEHS epidemiologist Andy Rowland that showed that female dental hygienists, who are exposed to mercury quite often in preparing fillings, had more problems conceiving than [women who were not exposed]. So, in order to study the mechanism of this effect, we tried to duplicate those results in animals.” Of the results Morgan says, “We weren’t able to reproduce any of those effects in our animal model. [However], it may be that longer exposures to lower concentrations may result in reproductive toxicity.” The research paper on this work is currently in press.

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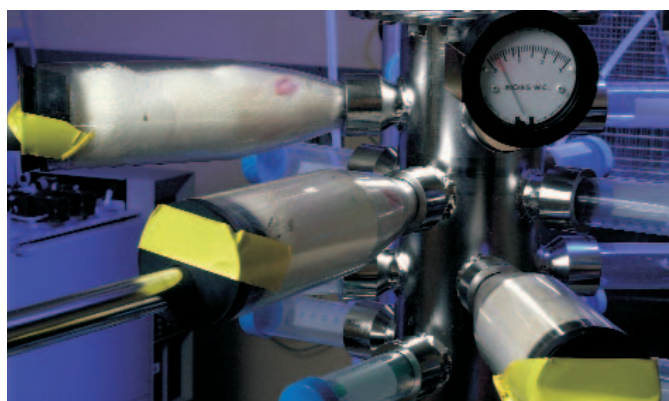
Still, the group continues to worry about exposures to mercury vapor. Besides exposures from fillings, mercury is also released as an emission from coal-fired power plants. Currently, the group is exploring how mercury inhaled by pregnant women is transferred to the fetus. In particular, they want to determine how much mercury makes its way into the fetal brain and, once there, if it can create behavioral problems. In studies, the group is exposing pregnant rats to various concentrations of mercury vapor and then examining mercury concentrations in various tissues. Although the project is ongoing, Morgan says, “We have detected considerable amounts of mercury getting through the placenta to the fetus. This is a potential problem.” Nevertheless, he



(l-r) Michael Moorman, Cassandra Shines, Dan Morgan.



Breath tests. For whole-body exposure studies, test animals are placed in cages that are then sealed inside a large chamber, which is filled with gases (l). Nose-only studies utilize a tree-like device (r) that delivers vapors directly to the animals' respiratory systems.



Steve McCaw/Image Associates, Inc.

adds, the studies have not shown much evidence of ensuing behavioral problems as a result of such mercury exposure, but data are still being analyzed.

Much of the concern about mercury surrounds a troubling characteristic of the element: once it is inhaled, it accumulates in the body and stays there. Perhaps worse yet, it often collects in the brain or other nervous system tissue. The question is whether it's possible then to eventually rid the body of it. Some practitioners of alternative medicine claim that various compounds, including glutathione and vitamin C, can bind mercury and eliminate it from the body. To test these claims, the Respiratory Toxicology Group has exposed rats to concentrations of mercury vapor that lead to high concentrations in the brain, and then treated them with various combinations of glutathione and vitamin C and with 2,3-dimercaptopropane-1-sulphonate, a chelator that is known to reduce the amount of mercury in the body. According to Morgan, it's too early to comment on the results from these studies.

Connections to Disease

The group examines toxicants other than mercury, as well. For example, recent studies have focused on styrene, α -methylstyrene, and divinylbenzene, chemicals that are used in the plastics industry. After exposing mice to these compounds, the investigators found that styrene and divinylbenzene caused toxicity in the nasal cavity and liver, but α -methylstyrene did not prove toxic at the concentrations tested.

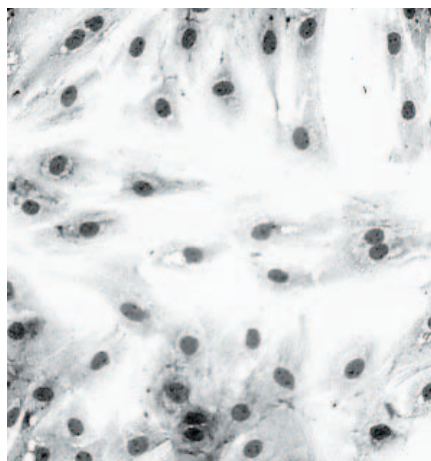
Moorman is using data from these studies to develop a physiologically based pharmacokinetic model—specifically, a computer simulation of the concentrations of the inhaled compound and its metabolites in an animal. The metabolism of these three compounds includes a step in which reactive metabolites formed in the liver are detoxified by conjugation with glutathione.

To model this metabolism, a simulation must calculate how much glutathione is in the liver, which is not an easy task. Glutathione concentrations change with the time of day, the rate of synthesis, and the rate of efflux from the liver, and through loss to reactive metabolites. Says Moorman, "When you try to model this in a computer to run a simulation, you need some way of calculating at each moment how much glutathione is there so that you can calculate the rate of reaction. Since that depends on several different variables, it presents a more difficult problem." Using data from experiments run by the group plus data from more than 100 reference sources, Moorman hopes to create a realistic model of the metabolism of these compounds. Once he is able to create a model that produces credible data, Moorman says, other people who are interested in risk assessment will then be able to use the same model to assess exposure in animals. For example, he says, "The treated animals may have exhibited some evidence of toxicity such as tissue lesions, biochemical changes,

or behavioral changes. Models can be used to identify what correlates with the development of toxic end points. The people in risk assessment can use information from these models to develop similar models to simulate what might happen in people, which of course is the whole point of this."

Members of the Respiratory Toxicology Group often collaborate with other investigators. Morgan has been working with NIEHS pulmonary biologist James Bonner for the past few years on vanadium pentoxide, a metal used in the petrochemical industry. According to Bonner, this metal is a source of occupational asthma in the petrochemical industry, and trace amounts of vanadium pentoxide are thought to be important to the toxicity of air pollution particulate matter. When Morgan and Bonner exposed rats to vanadium pentoxide, it caused fibrosis (the buildup of scar tissue) in the lungs and many other features of asthma. Says Bonner, "Perhaps the most significant finding so far is that we defined the importance of two receptors—the platelet-derived growth factor receptor and the epidermal growth factor receptor—to the progression of lung fibrosis and showed that blocking these receptors with specific tyrosine kinase inhibitors reduced the fibrotic effect of vanadium pentoxide in rats." He adds, "In general this offers a potential strategy for treating patients with fibrotic lung disease by suppressing the growth of lung fibroblasts, the major cell type that contributes to collagen deposition or tissue scarring."

In many if not most instances, toxicologists do not yet know which inhaled compounds can cause health problems or, for those that do, how they work. And effective treatments are currently lacking for many inhaled toxicants. As a result, there's an urgency to the work of the Respiratory Toxicology Group as it seeks to understand the air we breathe in the hopes of making it safer. —Mike May



Respiratory Toxicology Group

Direct impact. Work with rats to suppress the growth of lung fibroblasts, which lead to tissue scarring, has resulted in a potential strategy for treating fibrotic lung disease in humans.