New Data Help Toxicologists Home In on Assessing Risks

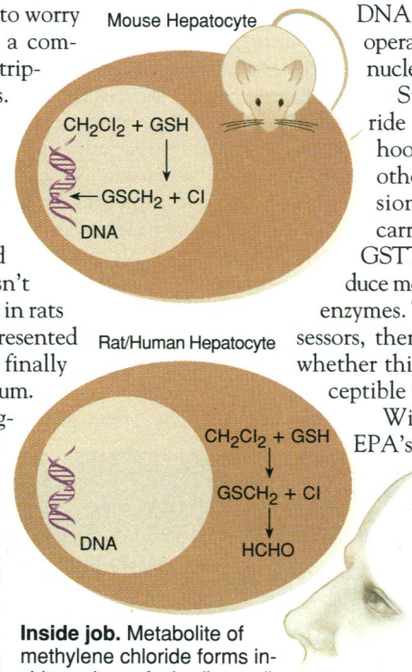
ANAHEIM, CALIFORNIA—More than 4000 toxicologists gathered here last month for the Society of Toxicology's 35th annual meeting. Participants learned about new clues to how pollutants such as nitrogen dioxide may contribute to asthma and other respiratory diseases, and why methylene chloride, a common industrial chemical, may be more of a threat to mice than to humans.

Methylene Chloride's Cancer Tricks

There are plenty of reasons to worry about methylene chloride, a compound widely used in paint strippers and industrial processes. It's toxic, and exposure to its vapors can cause neurological problems such as dizziness and numb fingers. But oddly, although the chemical causes lung and liver tumors in mice, it doesn't seem to increase cancer risk in rats and people. New research presented at the toxicology meeting finally helps to explain this conundrum.

The findings, which suggest that mice are unusually susceptible to methylene chloride because they have high levels of enzymes that interact with the chemical, could provide an early test case for new cancer assessment guidelines to be unveiled this spring by the Environmental Protection Agency (EPA). The EPA now classifies methylene chloride as a probable carcinogen because of the mouse results, but the new guidelines require regulators to take into account mechanistic and biological data as well as animal studies (*Science*, 21 April 1995, p. 356). As a result, the data could provide a reason to remove that carcinogen label. "We now have an excellent database for that kind of evaluation," says Trevor Green of Britain's Zeneca Central Toxicology Laboratory.

The EPA reduced its ranking of methylene chloride as a carcinogen after research in the 1980s suggested it didn't cause cancer in rats or humans, and at the meeting, Green and colleagues described data that may explain why. Methylene chloride causes tumors in mice, the group suspects, because it metabolizes the chemical via a glutathione pathway that is much more active in mice than in humans or rats. What's more, in mice the pathway's enzymes, glutathione-s-transferases (GST), are concentrated in the nuclei of lung and liver cells, where their metabolites easily inflict DNA damage. The enzymes operate mainly outside the nuclei of rat and human cells.



SOURCE: T. GREEN

Inside job. Metabolite of methylene chloride forms inside nucleus of mice liver cells *(top)*, where it can damage DNA and cause tumors, but outside nucleus of human and rat cells *(above)*.

Still, methylene chloride is not entirely off the hook as a carcinogen. As other findings at the session showed, some people carry two copies of a gene, GSTT1, that makes them produce more glutathione pathway enzymes. The task facing risk assessors, therefore, is to determine whether this subpopulation is susceptible to tumors.

William Farland, head of EPA's risk assessment office, says that next year the agency may begin drafting an addendum that could downgrade methylene chloride's risk level based on studies such as those presented at the meeting. Farland says, however, he won't know what the bottom line will look like "until we actually do the analysis."

Linking Asthma and NO_2

If you live in a smoggy city, you're familiar with nitrogen dioxide (NO_2)—it's the component of vehicle exhaust that makes smog brown. NO_2 also turns up in homes with gas stoves and kerosene heaters, and it's a suspected culprit in a recent, puzzling rise in asthma cases. But researchers had only circumstantial evidence of the role of NO_2. New results from rats, however, provide direct evidence implicating NO_2, along with hints that it may do its dirty work by activating components of the immune system and making the lungs more susceptible to allergens.

Ian Gilmour of the University of North Carolina, along with Dan Costa and Mary Jane Selgrade of the Environmental Protection Agency in Research Triangle Park, was trying to supplement the epidemiological evidence linking asthma and NO_2. For example, there are more hospital visits for asthma in periods of high levels of air pollution. Children in homes with gas stoves or kerosene heaters have been found to have more respiratory illnesses, and a recent study showed that British women who use gas stoves tend to wheeze and have shortness of breath more often than women with electric stoves.

To see whether NO_2 might really be at fault, the group first created asthmatic rats by injecting the animals with a solution containing house dust mite, a common asthma antigen, and *Bordetella pertussis* bacteria, which promote an allergic response. They then exposed rats to NO_2 at five parts per million for 3 hours—a level about 10 times higher than a human would ordinarily encounter but much lower than in a few previous studies. Then Gilmour's group sprayed a solution of house dust mite parts into the rats' lungs to trigger an asthma attack and re-exposed them to NO_2.

The results: The rats that breathed NO_2 were more sensitive to dust mite than were control rats given air. They had higher levels of dust mite antibodies in their blood and more allergen-activated lymphocytes in their spleens and lymph nodes. The NO_2-exposed rats also produced more inflammatory cells in the mucous of their lungs. Recently, Gilmour's team has also begun finding that the rats have lowered lung function, a clinical measure of asthmatic symptoms. "This suggests that NO_2 is making people [with asthma] more sensitive," says Richard Schlesinger of the Institute of Environmental Medicine and New York University Medical Center.

The experiments don't say exactly how the NO_2 exposure might be worsening the rats' asthma, but Gilmour says that NO_2 is already known to increase lung permeability, which might make it easier for dust mites to penetrate. In addition, he says, NO_2 may make the rats more allergic by affecting their population of T helper cells, immune cells that fall into two categories, T_H1 and T_H2. When the T_H1/T_H2 ratio gets skewed toward T_H2, the result is greater sensitivity to allergens. Gilmour says that may be happening in the NO_2-exposed rats, as their blood contains higher levels of IgE, an antibody produced in the T_H2 pathway.

To pin down the mechanism by which NO_2 affects the immune system, the group is now tagging dust mite antigen with radiotracers. The tracers will allow the researchers to compare how the allergen moves from the lungs to other cells in the body in NO_2-exposed and control rats. "We want to know where it goes and how quickly," says Gilmour.

–Jocelyn Kaiser