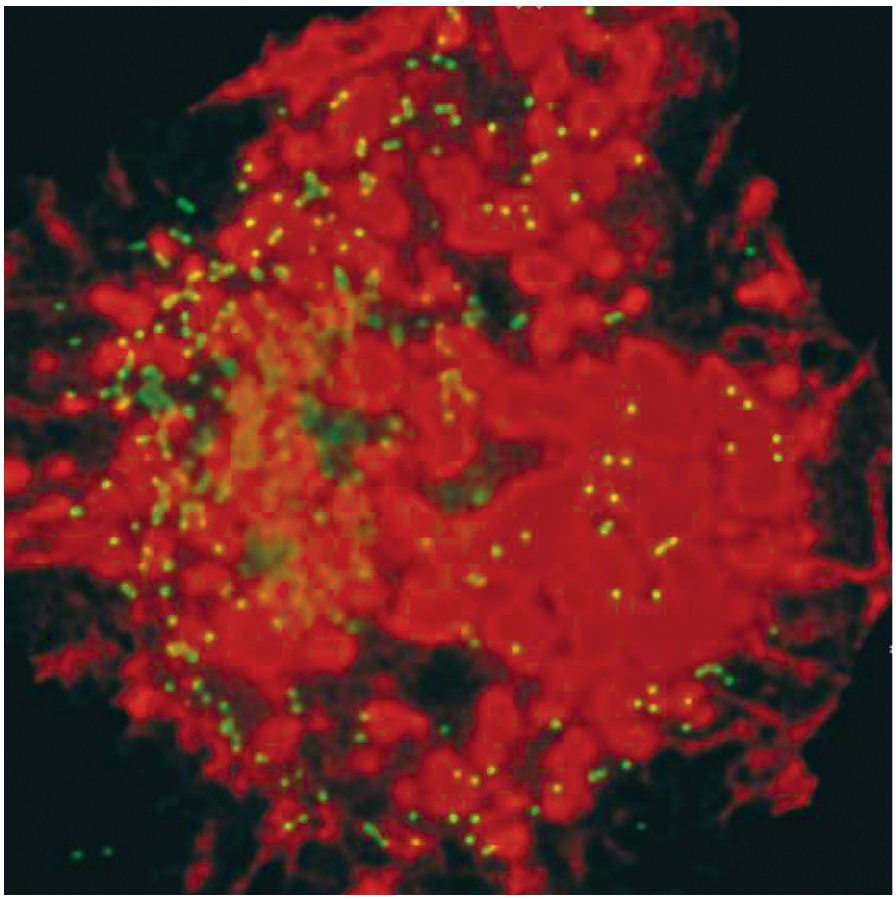
Particles in Practice

How Ultrafines Disseminate in the Body

Ultrafine particles (UFPs), those less than 100 nanometers in diameter, have existed for millennia in natural settings. But with the significant increase in UFPs resulting from human activities in the past few centuries (largely through combustion processes) and the potential for a deluge of nanoparticles as that industry gears up, are ancient human bodily defenses up to the substantial new hazards they now face? Findings by a team of Swiss, German, and Canadian researchers suggest that animals may be largely defenseless against the rapid dissemination of UFPs into cells throughout the body [*EHP* **113:1555–1560**]. Their findings, which include the first evidence of how individual particles are distributed within the lung, raise some concerns, especially since UFPs often end up in locations within cells where the tiny particles can impair many cellular functions.

General knowledge about the rapid penetration of UFPs into various body organs has surfaced in the past few years, but the specific distribution and mechanisms remain largely unknown. To explore the distribution, the research team performed two parallel sets of experiments.



Ultrafine infusion. A series of recent experiments demonstrates that ultrafine particles are widely disseminated in a variety of cells. A micrograph of one such experiment shows 0.2-micrometer fluorescent polystyrene particles (green) taken up by a macrophage (red).

Geiser et al.

In the first set of experiments, they investigated the spread of titanium dioxide UFPs in rats after a 1-hour inhalation of an aerosol containing the material. The team then evaluated lung tissue taken from the rats either 1 or 24 hours after inhalation.

They found that on average, 24% of the inhaled titanium dioxide they detected had penetrated cells throughout the lung and the bloodstream just 1 hour after inhalation. Within cells in different lung compartments, there was no difference in the 1-hour and 24-hour samples, suggesting that UFPs can easily move between compartments. The team continues to investigate what happens with the remaining 76% of the particles and with those that enter the bloodstream. There is evidence the particles spread throughout the body.

Of the particles they did find, 79.3% lodged in cells on the inner surface of airways and alveoli, 11.3% were within capillaries, 4.8% were within connective tissue, and 4.6% were within epithelial or endothelial cells. The researchers were surprised to find that most of the particles in the cellular cytoplasm were not attached to the membrane, as would have been expected if the particles had been encapsulated through endocytosis or phagocytosis. Floating in the cytoplasm, the particles can access many of the structures within the cell, such as the nucleus and mitochondria, increasing the potential toxicity of the particles.

In the second set of experiments, the researchers explored the movement of three sizes of fluorescent polystyrene UFPs and of gold UFPs after the particles were introduced to cultures of swine macrophages and human red blood cells. They found all three particle sizes (1.0, 0.2, and 0.078 micrometer) penetrated the swine macrophages, though in perplexingly different proportions—only 21% of the macrophages contained the medium size, while 77% contained the smallest and 56% contained the largest. In human red blood cells, they found the smallest and medium sizes, but not the largest.

The experiments did not offer evidence about exactly how the tiny, insoluble particles disseminate so extensively and rapidly into so many different cells, but the researchers note that other experiments have demonstrated a number of possible mechanisms. The researchers also note their findings are specific to just the few substances they studied, and differ in some ways from those for iridium, one of the few other materials evaluated in some detail. **–Bob Weinhold**

Testing the Additivity Assumption

Chemical Mixtures and Thyroid Function

It is well established that many environmental contaminants can disrupt thyroid hormone (TH) homeostasis, which is vital during fetal development and for a variety of physiological processes in adults. Among known TH disruptors are polychlorinated biphenyls (PCBs), dioxins, and dibenzofurans, all members of the polyhalogenated aromatic hydrocarbon (PHAH) chemical family. Little is known, however, about how mixtures of such chemicals at typical environmental exposure levels may disrupt TH functions. Nor is it clear whether effects are additive, synergistic, or antagonistic—that is, whether there is interaction between constituent chemicals, whether their cumulative influence is more than the sum of its parts, or whether they cancel each other out. With respect to risk assessment, the U.S. Environmental Protection Agency's default assumption is that the effects of chemicals in

mixtures are additive. Now a team of researchers has tested the additivity assumption and found that it is relatively robust at exposure levels typical for humans [*EHP* **113:1549–1554**].

Over a four-day period the team exposed young female rats to six different doses of a combination of 18 PHAHs comprising 2 dioxins, 4 dibenzofurans, and 12 PCBs. The team determined dose–response information for each constituent chemical before the mixture was tested. The concentration of each chemical in the mixture reflected typical concentrations measured in breast milk and in fish and other foods. The mixture was also formulated so that even at the highest mixture doses, the rats' exposure to each constituent chemical was at or below the known no-observed-effect level for that chemical.

The mixture reduced the rats' serum thyroxine (T_4; the most common form of circulating TH) in a dose-dependent manner. At lower doses the effects were additive. At higher doses T_4 declined by as much as 50%, and the effects were mildly synergistic—about twice what was predicted by additivity—so that even in the upper range the effects as predicted by the additivity hypothesis came close to actual results.

Significantly, the study also showed that the mixture exerted an effect on T_4 even though concentrations of its constituent chemicals were at least an order of magnitude below their known effective doses. This indicates that considering individual chemicals in isolation may not predict their effects in mixtures because, even though chemicals may not be potent enough by themselves to cause effects, the cumulative effects of low doses of many chemicals may be enough to do so.

The multiple functions of TH, such as its role in fetal development and its regulation of metabolism and heart rate, make it vulnerable at many points. The team estimates that there could be as many as five distinct mechanisms by which chemicals exert antithyroid effects for which a reduction in circulating T_4 is the common end point.

Several factors temper the study results. One is that this study was a series of short-term exposures that did not encompass all the chemicals' varied half-lives. The results therefore cannot be directly extrapolated to the effects of chronic exposures and may be subject to confounding by pharmacokinetic differences. Another is that thyroid disruption mechanisms in rats may not be identical to those in humans. The team is now working on testing how a more complex chemical mixture may interact with dietary iodine insufficiency to produce thyrotoxic effects. **–Valerie J. Brown**

Cadmium and Kidneys

Low-Level Exposure and Effects in Women

Widespread exposure to the heavy metal cadmium occurs through both natural and industry-related sources. The general population is likely to encounter low-level chronic exposure through smoking and from dietary sources, particularly shellfish, grains, and vegetables. In 1999 an ongoing population-based Swedish study, Women's Health in the Lund Area, was expanded to include low-level cadmium exposure. Analysis of the data collected now reveals a small but significant kidney response to low-level cadmium exposure [*EHP* **113:1627–1631**]. This suggests that low-level cadmium exposure may pose a significant public health risk.

Owing to extremely slow excretion, cadmium accumulates in the body, especially in the kidneys. Kidney damage is the primary consequence, but most toxicity data are from exposures in occupational settings or severely polluted areas. The effects of low-level exposure are less certain.

A primary function of the kidney is to filter excess water and metabolic by-products from the blood for urinary excretion. This filtration occurs in more than 1 million nephrons, each of which contains a blood capillary (the glomerulus) intertwined with a urine-collecting tubule. In the current study, researchers assessed glomerular and tubular fitness by measuring kidney function markers in blood and urine, respectively. Blood testing also revealed ongoing cadmium exposure, and urinalysis indicated cadmium body burden.

The team analyzed data, including blood and urine samples, collected from 820 women aged 54–63 years. Blood levels of creatinine and cystatin C were measured in 742 participants to calculate glomerular function. Urinary concentrations of calcium, human complex-forming protein, and *N*-acetyl-β-D-glucosaminidase—all markers of tubule function—were available for 813 women. The researchers additionally collected data on medications taken, smoking history, lead exposure, and incidence of diabetes and hypertension to control for potential confounding factors.

Cadmium concentrations were similar or slightly higher compared with previous data from Sweden and much lower than concentrations reported for populations in highly polluted areas in Europe and Japan. Current or former smokers had cadmium concentrations

Cadmium connection. A new study shows that kidneys respond to even low-level chronic cadmium exposure such as that obtained from smoking and eating grains.

Photodisc

that were 90% higher in blood and 40% higher in urine than concentrations measured in participants who never smoked. Consequently, multivariate analyses were conducted on data from all participants as one group and from those who had never smoked as another group.

Cadmium concentrations were positively associated with the tubular function markers, indicating some damage to the tubules. Increased cadmium was also associated with decreased creatinine clearance, reflecting a reduced glomerular filtration rate. The lowest-observed-effect level for increased tubular markers was a mean urinary cadmium concentration of 0.6 microgram per liter, which is lower than previously reported. A reduction in glomerular filtration rate was associated with a minimum mean urinary cadmium concentration of 0.86 microgram per liter.

The researchers speculate that effect levels might be even lower for people with diabetes, a disease carrying high risk of kidney damage similar to that caused by cadmium exposure. Although the effects of low-level cadmium exposure are clinically minor, they should be viewed as early indicators of potential severe health effects, according to the researchers. Given the size of the exposed population, there may be a significant public health risk, and efforts beyond smoking cessation programs are needed to reduce exposure. **–Julia R. Barrett**

Indoor Air Complaints

VOCs May Not Be Cause of Acute Effects

Over the past few decades, researchers have been trying to pin down the specific chemical culprits behind increasing complaints of poor air quality inside offices and other buildings. Among the many chemicals suspected so far have been volatile organic compounds (VOCs) and ozone, prominent pollutants in indoor environments. But VOCs alone, or in combination with ozone, may not be the prime source of acute health problems, says a team of New Jersey investigators [***EHP* 113:1542–1548**]. Instead, they found that psychological stress was a more salient factor, but they acknowledge that a number of limitations in their study preclude applying this finding to all indoor air complaints.

The study investigated the short-term acute health effects of exposure to ozone, a mixture of 23 VOCs, and stress. The research was conducted in a controlled chamber into which either a relatively high level of the VOC mixture (26 milligrams per cubic meter), the VOCs plus moderate concentrations of ozone (40 parts per billion), or clean air with a low one-minute spike of VOCs (about 2.5 milligrams per cubic meter) was introduced. In the middle of each three-hour test session half of the volunteer subjects had to make a four-minute speech on a controversial subject as a stress test, while the other half performed simple arithmetic problems. The test sessions were held one week apart.

The researchers evaluated stress by measuring cortisol secretions in saliva. To assess health effects, they evaluated selected performance measures, as well as 33 observed and self-reported physical and behavioral indicators, such as headache, nausea, eye irritation, nervousness, and leg cramps.

They found the challenge of public speaking induced a significant increase in the subjects' measures of stress. However, even with that increase in stress, no significant increase in health symptoms or reduction in neurobehavioral performance was linked to the exposures to VOCs either alone or combined with ozone, despite sharp increases in many secondary pollutants resulting when ozone was added to the VOC mixture.

The 130 female volunteers exposed to each air mixture constituted the largest group evaluated in a study of this kind, and the researchers determined the numbers were of sufficient power to produce significant findings. However, all the subjects were healthy, young (mean age 27.2 years), and well educated (mean education of 15.2 years), demographically limiting the applicability of the findings.

In addition, the team acknowledges that its testing, while extensive, didn't represent many aspects of a typical office building. For instance, the test chamber did not include carpet, many office furniture materials, and other normal interior accoutrements that might interact with VOCs and ozone. The mix of VOCs, although extensive, likely didn't represent the mix in many buildings. Further, the ventilation rate in the test chamber was substantially higher than in many buildings at which complaints have been lodged.

Further, the public speaking challenge, although successful at inducing stress, wasn't representative of the multiple complex stressors experienced in a typical work day. And the testing period was very short, providing no information on the potential chronic effects that may be induced by longer-term exposures to chemicals and stress. Nonetheless, the findings are helpful in pinning down the relative contribution, or lack thereof, of certain mixtures and concentrations of VOCs and ozone to poor indoor air quality. **–Bob Weinhold**

Maybe it's nerves. A study of indoor air pollutants and stress (induced by public speaking) shows that stress, not VOCs, may be a larger culprit behind sick building complaints.

Photodisc