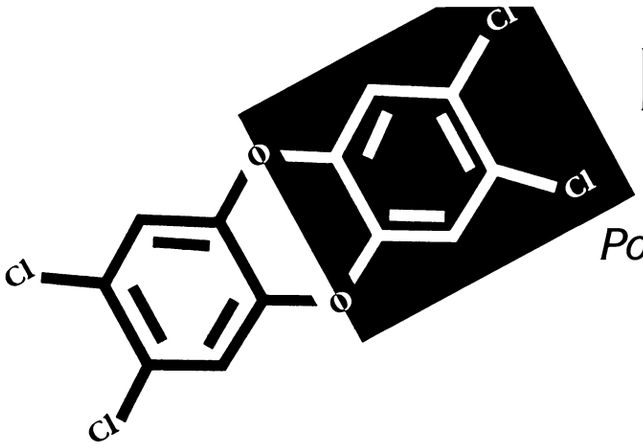


Dioxin's Other Face

Portrait of an "environmental hormone"

By KAREN F. SCHMIDT

When a villain starts looking like a friend, it's time to look again. Take TCDD, the most notorious and potent member of the dioxin family. Once demonized as "the most toxic synthetic chemical known to man" because of its exquisitely lethal effect on guinea pigs, TCDD now appears "no more risky than spending a week sunbathing," as a recent New York Times article put it.

In 1983, scares over TCDD forced several thousand residents of Times Beach, Mo., to permanently flee their tainted community. But "given what we now know about this chemical's toxicity and its effects on human health, it looks as though the [Times Beach] evacuation was unnecessary," Vernon N. Houk — the scientist at the Centers for Disease Control who originally spearheaded the evacuation — acknowledged, according to the Times article last August.

Most dioxin researchers now suspect that only very high doses of TCDD — as occur accidentally or in certain occupational settings — may increase the risk of cancer in humans. But that redefinition does not necessarily imply that the chemical is harmless at lower doses.

Indeed, this near-ubiquitous contaminant — a by-product of the paper, wood and herbicide industries and of the incineration of organic solvents — is gaining a new and nasty reputation among toxicologists: as an "environmental hormone" that subtly disrupts normal physiology in ways not completely understood. More potent than some of the body's natural chemical messengers, TCDD suppresses the immune system of mice at least 100 times more effectively than corticosterone, a hormone known for that effect, dioxin researchers say. In fact, increasing evidence suggests that TCDD's ability to mess with the immune system — not its carcinogenicity — may represent its greatest threat to public health.

All this flip-flopping on the chemical's toxicity may puzzle the public, but it has proved no less confusing to dioxin researchers. TCDD's toxic deeds result from a perplexing web of interactions. Unlike most toxicants, dioxin causes an array of biological responses that vary widely according to tissue. For example, TCDD may goad one cell type to reproduce wildly and cause another to deviate from its normal path toward specialization.

Different animal species also vary in their responsiveness to dioxin. It takes several thousand times more TCDD to kill a hamster than it does to kill a guinea pig. Yet the hardy hamster is quite susceptible to TCDD's triggering of increased cellular levels of a P450 enzyme — a protein catalyst that plays a role in detoxifying certain chemicals within the body and rendering others more toxic.

Unfortunately, epidemiologic studies have done little to resolve toxicologists' muddy understanding of dioxin's human hazards. For instance, such studies rarely turn up consistent adverse effects among humans exposed to dioxin — with the exception of chloracne, the disfiguring skin eruptions associated with acute TCDD exposures.

Consider studies of U.S. troops potentially exposed to Agent Orange, a TCDD-tainted herbicide, while serving in Vietnam. An Air Force study of veterans who had participated in the Ranch Hand defoliation program found indications that these men faced an increased — though statistically insignificant — risk of skin, genito-urinary and otopharyngeal cancers and a tendency to develop underactive thyroids and diabetes (SN: 3/3/84, p.132). Another study found an increased incidence of high blood pressure, benign fatty tumors, sensitivity to light, and depression among these veterans and miscarriages among their wives (SN: 11/19/88, p.325). A third study found that Vietnam veterans suffer higher-than-normal rates of non-Hodgkins lymphoma, a deadly cancer of the lymph nodes, but it failed to tie the disease to Agent Orange exposure (SN: 4/14/90, p.236).

"If you think of TCDD as a hormone, it makes it easier to understand these very big differences," asserts Linda S. Birnbaum, director of environmental toxicology at the Environmental Protection Agency's Health Effects Research Laboratory in Research Triangle Park, N.C. A single hormone can induce an array of effects in different tissues and species, she explains.

The environmental hormone theory also helps explain why dioxin appears to induce a variety of cancers rather than a single hallmark type — such as the rare form of cancer, called mesothelioma, that signals asbestos exposure. Unlike most carcinogens, TCDD does not directly damage DNA in a target organ, notes George W. Lucier of the National Institute of Environmental Health Sciences in Research Triangle Park. However, he explains, dioxin clearly enhances abnormal cell growth and appears to cause cancer by amplifying the diverse activities of other carcinogens.

Two recent epidemiologic studies support the human carcinogenicity of TCDD, at least at fairly high doses. In one, researchers at the National Institute for Occupational Safety and Health examined health records for workers exposed to TCDD at a dozen chemical plants. Overall, the 5,172 workers appeared 15 percent more likely to die from cancer than the general population, Marilyn A. Fingerhut and her co-workers reported in the Jan. 24, 1991 New England Journal of Medicine. However, records on the 1,520 workers whose exposures began at least 20 years ago — when plant dioxin levels were typically much higher than today — showed nine times the normal rate for one particular cancer, soft-tissue sarcoma.

A similar study of 1,583 pesticide-plant workers in Germany showed that, compared with the general population, TCDD-exposed workers experienced a 24 percent higher rate of death from all cancers. Among workers with more than 20 years' exposure, the cancer death rate increased to 87 percent above normal, according to Alfred Manz and his co-workers at the Center for Chemical Workers' Health in Hamburg. However, they reported in the Oct. 19, 1991 Lancet, the increases were not linked to any one

Science Service, Inc. is collaborating with JSTOR to digitize, preserve, and extend access to *Science News*. www.jstor.org

particular type of cancer.

On the basis of these and other studies, Birnbaum says, "I really feel that high-dose exposure to dioxin has the potential to cause cancer." However, she adds, "I'm very concerned that much lower exposure to dioxin may result in adverse health effects that are very subtle and difficult to detect."

In an effort to update federal regulatory guidelines for human exposures to dioxin – now considered a "probable human carcinogen" – EPA has begun reassessing the scientific data on dioxin. In its draft version of this document, due in June, EPA will focus much greater attention on toxicological data revealing TCDD's reproductive, developmental and immunotoxic effects. This document will also establish TCDD as the first pollutant to be regulated on the basis of toxicity observed at the cellular level.

Now that most dioxin researchers believe a single fundamental mechanism underlies all of TCDD's effects (see box, p. 26), toxicologists such as Lucier can construct a unifying mathematical model to describe how dioxin triggers biological effects in cells and organisms. Others, including Birnbaum and Nancy I. Kerkvliet of Oregon State University in Corvallis, will help flesh out the model by collecting specific data on the dose-response relationships between TCDD and its array of biological effects.

"Dioxin is no more and no less potent than it ever was," Kerkvliet says. "But understanding the mechanism can now help us better estimate the human risk."

So far, studies in mice suggest that dioxin's immunotoxic punch occurs in extremely low doses and may well be more important than cancer in determining dioxin's primary health risk, adds Birnbaum. At least in animals, some suppression of immunity consistently occurs at TCDD doses lower than or equal to those required for triggering increased production of a P450 enzyme – previously considered a liver cell's most sensitive response. In fact, Birnbaum's preliminary unpublished data suggest that immunotoxicity in mice could be occurring at TCDD doses 1/15 of that needed to boost levels of this enzyme, she says.

Even though scientists continue to debate whether an excess of this P450 enzyme causes any adverse health effects, "few people will contend that suppression of the immune system is not an adverse health effect," she observes.

To study TCDD's immunotoxicity, researchers generally use mice, whose immune systems model those of humans. In one typical test, EPA toxicologists exposed mice to TCDD, then injected them with a harmless, antibody-stimulating agent – red blood cells from sheep. An animal's ability to produce antibodies serves as one useful measure of its immunological health. Compared with normal mice, the TCDD-treated animals produced fewer antibodies to the sheep blood cells, Birnbaum says.

EPA researchers have also measured how well TCDD-treated mice respond to viral infections, such as influenza. Mice pretreated with dioxin readily die after exposure to a quantity of virus that rarely kills healthy mice, Gary R. Burleson of EPA's Research Triangle Park facility and his co-workers reported in the November 1990 JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH. Birnbaum's team is now trying to determine the dose-response relationships of these immunosuppressive effects.

Because "there are so many ways to cause immune suppression," Birnbaum explains, scientists can only speculate as to how TCDD weakens immunity. Indeed, she notes, "there could be multiple mechanisms."

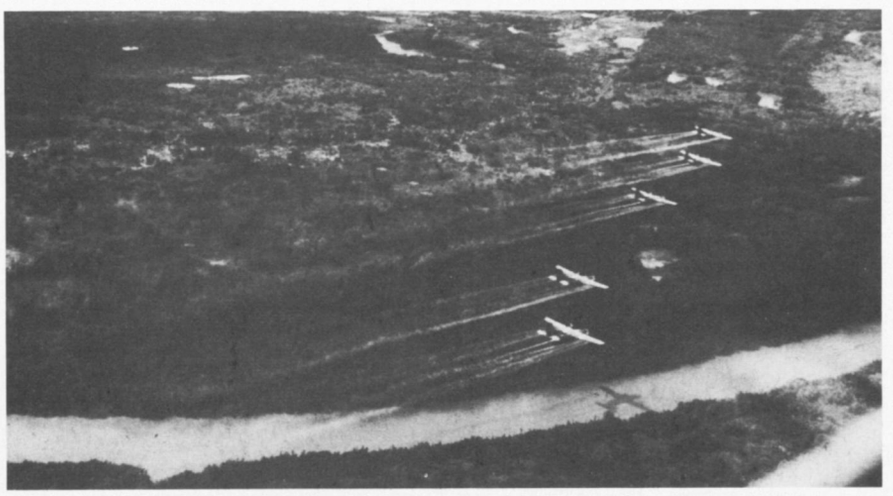
At a minimum, TCDD probably interferes with the normal influences of hormones on the immune system, Kerkvliet posits. She says that it appears TCDD can combine with a particular type of receptor protein inside a cell's fluid interior, and then inappropriately turn on specific genes. Some of the victimized cells may reside in glandular tissues, such as the thymus, where hormones influencing immunity are produced.

Dioxin also appears to act directly on the immune system, says Kerkvliet, who studies TCDD's effects on a group of white blood cells called T-lymphocytes. She and her co-workers were initially confounded when they observed that although TCDD boosts production of T-lymphocytes – which referee the total immune response – it still causes an overall decline in the mouse immune system's ability to fight foreign substances, be they viruses or pollutants.

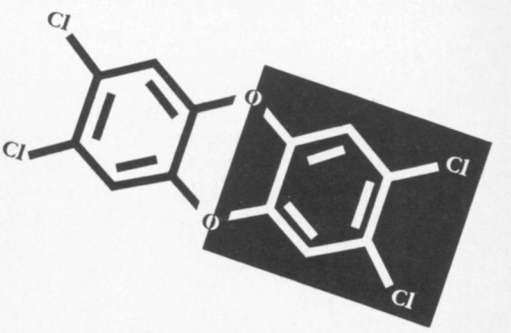
"We think TCDD is turning on certain T-helper cells inappropriately, which then makes the overall immune response suppressed," Kerkvliet now says. This idea fits with a new hypothesis that not all of the specialized T-lymphocytes called T-helper cells "help" strengthen the immune response; some may actually inhibit it, she notes.

For the most part, Kerkvliet believes that dioxin initiates its direct immunotoxic effects by binding to the dioxin protein receptor – perhaps in the bone marrow, where white blood cells are produced – and by toying with the normal functioning of genes. Recently, her research group studied how TCDD affects a mouse's production of cytotoxic T-lymphocytes, which destroy cells infected with viral invaders.

The team compared responses in TCDD-treated mice with normal and defective dioxin receptors, and found significantly greater immune suppression in the mice with normal receptors. They also compared the responses of these mice to a variety of polychlorinated biphenyls (PCBs), chemical relatives of dioxin. Immunity suppression indeed correlated with each chemical's ability to bind to the protein receptor, Kerkvliet's group reported in the April 1990 FUNDAMENTAL AND APPLIED TOXICOLOGY. These findings suggest that dioxin's protein receptor plays an important role in its immunotoxicity, they say.



U.S. Air Force



During the Vietnam war, the U.S. military dumped millions of gallons of TCDD-tainted Agent Orange over South Vietnam. Veterans who participated in this defoliation program, called Operation Ranch Hand, have experienced a variety of health problems that might be related to dioxin exposure.

Given the complexity of the immune system, however, not all dioxin researchers are ready to settle on a single receptor-based mechanism to describe all of TCDD's immunosuppressive effects.

Michael P. Holsapple of the Medical College of Virginia/Virginia Commonwealth University in Richmond has also observed that "when we give dioxin to animals or white blood cells, we see problems with their immune function." However, he adds, "the immune system is probably just a microcosm of the whole complex story for dioxin." He suspects that TCDD may employ different routes of attack depending on the conditions of exposure, he says.

For instance, his team compared the effects of acute versus chronic TCDD exposures on the ability of mice to produce antibodies to sheep red blood cells. After a single acute dose, mice with normal dioxin receptors suffered greater immune suppression than mice who had defective receptors. However, when mice received this same amount of TCDD over a two-week period, both mouse strains showed similar immunosuppressive responses, he and his colleagues report in the January 1992 TOXICOLOGY AND PHARMACOLOGY. Holsapple now theorizes that TCDD's mechanisms may not always involve the receptors and may differ at high and low doses.

Throughout the developed world, humans already experience chronic low-dose exposures to dioxins, primarily through their diet (SN: 7/13/85, p.26). Holsapple and his co-workers suspect that people "exposed to low doses over an extended period of time (i.e. months to years) may be at increased risk to immunotoxic effects by these chemicals through additional and presently unidentified mechanisms."

One such mechanism can be inferred from developing research in the field of endocrinology, Holsapple says. Scientists had assumed that, much like dioxin, all steroid hormones act exclusively through an intracellular protein receptor that helps it target a particular gene (SN: 8/10/91, p.85). But Holsapple points to new evidence suggesting that some steroid hormones – including progesterone, estrogen and testosterone – can also bind to other receptors on the outside of a cell membrane, where they can regulate the flow of salts into and out of a cell. TCDD might also tinker with a cell's physiology through such a mechanism, he suggests.

Dioxin's Cellular Siege

Dioxin may cause everything from immune suppression and liver tumors to cleft palate in mice, but all of these adverse effects begin with the same initial cellular changes, most dioxin toxicologists now believe.

This "new" view – the impetus behind the Environmental Protection Agency's (EPA) current reassessment of dioxin's risks – actually traces back to 1976. That year scientists reported discovering that TCDD – the most toxic and best studied of the 75 dioxin species – binds with a receptor protein residing in the cells it invades. Only recently, however, did a group of 38 international dioxin experts unanimously conclude that *every one* of TCDD's myriad effects appears to begin with the compound's binding to this receptor – a mechanism resembling that of the body's own steroid hormones.

"Those biological responses [to TCDD] that have been examined in great detail have all been shown to involve this receptor," says EPA toxicologist Linda S. Birnbaum, one of the scientists who reached agreement at the dioxin conference held at Cold Spring Harbor Laboratory (N.Y.) in late 1990. She says EPA hopes to base a new assessment of human health risks from dioxin – and new regulations – on the recently recognized universality of this receptor in TCDD's effects.

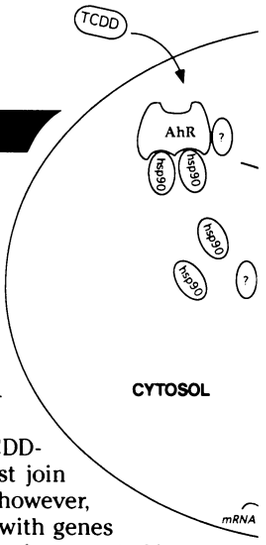
In the 15 years since scientists first realized that dioxin binds to a receptor, called aryl hydrocarbon (Ah), they have developed a detailed picture of how TCDD acts on individual cells. For example, the Ah receptor actually comprises several proteins that cluster together in the liquid interior of most cells in the body. Once dioxin seeps into a cell and links up with these proteins, the TCDD-protein complex can enter the cell's nucleus and cause trouble by meddling with the on-off switches of genes.

Cells of some tissues, such as the liver, teem with Ah receptor proteins, while others may contain only a few. Why our cells should produce such receptors for dioxin remains a mystery.

Perhaps the body produces a hormone that normally operates through the Ah receptor, speculates Thomas A. Gasiewicz of the University of Rochester (N.Y.) School of Medicine. As scientists come to understand the similar and overlapping actions of our natural chemical messengers – hormones and neurotransmitters – with toxicants and drugs, traditional definitions are blurring, he says.

"Just because a compound binds to a receptor doesn't mean it's necessarily going to be toxic," Gasiewicz observes. Any natural hormone that binds to the Ah receptor probably plays a healthy role in regulating cell growth, he says. Even steroids – vitally important hormones that act through protein receptors – can turn "toxic" when their levels get out of whack, he adds. For instance, excess estrogen can lead to cancer.

Scientists have no clues as to the identity of the hormone that normally binds to the Ah receptor, but they assume it physically resembles TCDD, for which there's a perfect docking site on one protein subunit of the Ah receptor. Once TCDD enters the cell, it binds with the receptor and evicts other subunits, called heat shock protein 90.



The remaining TCDD-receptor complex must join yet another protein, however, before it can interact with genes in the cell's DNA, Gasiewicz reported in the March 19, 1991 BIOCHEMISTRY. This additional protein, called the Ah receptor transforming protein (Art), does not directly bind to TCDD, he found, but instead seems to enable the whole complex to hook up with DNA. Gasiewicz now theorizes that Art, which may vary slightly in structure according to the tissue, might steer the complex to act on certain genes.

To get at those genes, the TCDD-receptor complex must first enter the cell's nucleus. Although it's not clear just which events occur in the liquidy cytosol surrounding the nucleus, Oliver Hankinson of the University of California, Los Angeles, has found a protein that must join the complex before the ensemble can gain passage into the cell's center. This protein bears a basic helix-loop-helix structural motif common to DNA-binding proteins, Hankinson reported in the May 17, 1991 SCIENCE. In fact, he told SCIENCE NEWS, it may be the same Art protein that Gasiewicz discovered.

Although they are still identifying the receptor's protein players, Gasiewicz and Hankinson know that it takes at least two proteins and TCDD to create a

In mice, it takes far smaller quantities of TCDD to suppress immunity than it does to unleash most of TCDD's other toxic effects. And white blood cells in both mice and humans respond similarly to TCDD. But to date, there's little evidence to suggest that low-dose exposures to TCDD suppress immunity in humans. Birnbaum, Kerkvliet and Holsapple contend that studies of dioxin-exposed humans have asked the wrong questions.

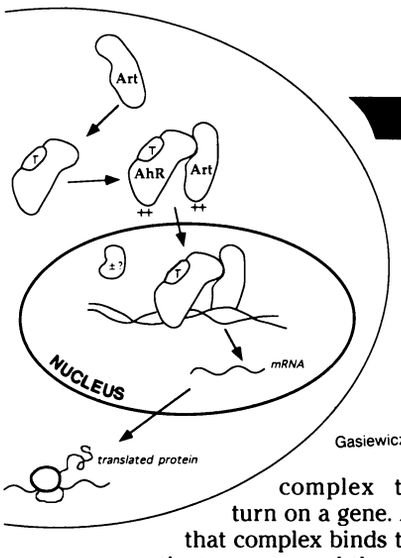
"If I were to take mice and ask the same [research] questions that are routinely asked of the populations at Times Beach, or in the Ranch Hand study, I would come up with a very nebulous picture [of TCDD's immunotoxicity]," says Holsapple. "But when we ask different questions [in mice], we can certainly show very strong effects on the immune response."

Birnbaum is now calling for a study that will determine how well TCDD-exposed people mount an antibody response to a novel antigen. Perhaps a new flu vaccine – one that uses an influenza strain that hasn't previously infected humans – can serve the function of the sheep red blood cells given to laboratory mice, she says.

But Kerkvliet says EPA shouldn't hold its breath waiting for the definitive epidemiologic study. It would be next to impossible to prove beyond a doubt that dioxin causes immune suppression in humans, she asserts. Unlike sheltered laboratory mice, people come in contact with many immunity-altering forces – such as stress, drugs and disease. Regulators should therefore base their limits for safe exposures to dioxin on animal models and on our developing scientific understanding of TCDD's mechanisms of action, she says.

Kerkvliet suspects that most Americans – who harbor about 30 parts per trillion (ppt) of dioxins in their blood, including about 7 ppt of TCDD – fall below the range of dioxin exposures that can jeopardize immunity. However, she adds, populations that commonly receive higher doses, such as nursing infants (SN: 4/26/86, p.264), chemical workers and people who consume large quantities of fish, could conceivably experience compromised immunity.

"The fact that you can't clearly show the effects in humans in no way lessens the fact that dioxin is an extremely potent chemical in animals – potent in terms of immunotoxicity, potent in terms of promoting cancer," says Kerkvliet. "I simply don't believe that humans represent some unique species." □



Once TCDD (T) seeps into a cell, it binds to an aryl hydrocarbon receptor (AhR) and kicks off the heat-shock protein (hsp90) subunits. The complex then joins an Ah-receptor-transforming protein (Art) and passes into the nucleus, where the ensemble binds to DNA and switches a gene on or off. Unidentified "mystery proteins" (?) may also participate throughout this process. An activated gene triggers production of messenger RNA (mRNA), the instructions that a cell then uses to build a specific protein, such as a P450 enzyme.

Gasiewicz

complex that can turn on a gene. And once that complex binds to DNA, it can activate a gene and thereby cause the cell to produce excessive quantities of a certain protein. Theoretically, dioxin could also turn some genes off, which can also cause ill effects.

Unlike the steroid hormones, which degrade in a few hours, TCDD molecules require seven years to reduce their concentration by half. Because of TCDD's long half-life, it appears that the body cannot regulate this process and the gene's "switch can be turned on for inappropriately long periods of time," Gasiewicz points out. Thus, one TCDD molecule can continuously disrupt normal cell physiology.

In developing a model to explain dioxin's cellular actions, scientists have primarily studied how TCDD turns on a gene for a P450 enzyme. While this specific enzyme normally helps the body excrete toxic substances, it sometimes renders them more potent instead. Though scientists don't know if increased levels of P450 enzymes contribute to any of dioxin's toxic effects, they do know that the TCDD-receptor complex probably flips the P450 gene switch by a mechanism that applies to many other genes as well.

"We're beginning to know the beginning of the story, which is how the receptor activates genes," says Hankinson. "And to some degree we understand the end product [why animals get cancer and why they die]. The real black box is *which* genes are turned on and how they relate to the biological effects of dioxin."

Recently, William F. Greenlee and his colleagues at Purdue University in West Lafayette, Ind. found several new genes targeted by the TCDD-receptor complex. In the Oct. 18, 1991 SCIENCE, they describe identifying two dioxin-responsive genes in human skin cells. The first directs the production of plasminogen activator inhibitor-2, a protein that functions in embryonic development, wound healing, inflammation and cancer. The second gene contains the code enabling a cell to produce cytokine interleukin 1-beta, a protein involved in inflammation and immune responses.

These are the first genetic targets of dioxin to be discovered since the P450 gene, and Greenlee says "these [new] genes are likely to play an important role in the toxicity of TCDD." He says they could plausibly be involved in chloracne – the hallmark skin reaction that usually signals acute human exposure to dioxin. These findings lend credence to a unifying mechanism for all of dioxin's diverse effects, Greenlee says.

"If you look at the broad range of events, it all comes back to a very generic process," he says.

Making the leap from a generic cellular mechanism to guidelines for human exposure – as EPA proposes to do – could prove tricky, however. Some toxicologists argue that receptor involvement implies a certain rate-limiting event – perhaps a minimum number of TCDD molecules needed to bind – before a cell or animal responds with a measurable change in its physiology. This in turn suggests that a "threshold" concentration may exist, below which dioxin causes little or no harm (SN: 5/18/91, p.308).

However, scientists should not assume a safe threshold exists, argues George W. Lucier of the National Institute of Environmental Health Sciences in Research Triangle Park, N.C. To date, his research team has found no predictable, consistent pattern in the dose-response relationships for a number of dioxin's toxic effects – nor evidence of any thresholds.

"My data might not prove that a threshold doesn't exist," Lucier concludes, "but there's also no evidence to support that one does exist."

Still, which ever way the chips fall, Lucier says he's pleased that EPA is finally attempting to incorporate recent research findings into an updated view of dioxin's human toxicity.

"A lot of dollars are spent doing mechanistic research," he comments. "There are thousands of papers on dioxin. We ought to be able to use some of that information in the risk assessment process." – *K. Schmidt*