

Aspartame Cancer Risks Revisited

Prenatal Exposure May Be Greatest Concern

Aspartame is an artificial sweetener used in more than 6,000 diet products, beverages, and pharmaceuticals. In March 2006, *EHP* published the first compelling experimental evidence for the carcinogenic effects of aspartame at a dose level within range of human daily intake [*EHP* 114:379–385; Soffritti et al.]. A second animal study by the same research team now indicates that the carcinogenic effects of aspartame are magnified when exposure begins during fetal life [*EHP* 115:1293–1297; Soffritti et al.].

The first study involved a much larger sample size than had been used in previous experiments. It showed a dose-related increase in the incidence of various malignant tumors in female rats fed aspartame from 8 weeks of age until natural death. The experiment was impressive for its long observation period and comprehensive assessment of aspartame's carcinogenic potential. Nevertheless, as the researchers acknowledged, the study did not take into account prenatal or perinatal exposures.

In the new study, the investigators added aspartame to the standard diets of male and female Sprague-Dawley rats from the twelfth day of fetal life until natural death. Rats were fed in groups of 70 to 95 each at aspartame concentrations of 2,000, 400, and 0 ppm—approximately

equivalent to a daily intake of 100, 20, or 0 mg/kg body weight. The current limits for acceptable daily intake are set at 50 mg/kg body weight in the United States and 40 mg/kg body weight in Europe.

The researchers report that rats fed at the 400 ppm level showed nonsignificant increases in malignancies. For animals fed at the 2,000 ppm level, there was a significant increase in the incidence of lymphomas/leukemias and malignant mammary tumors. Furthermore, compared with the team's earlier study in which animals were dosed postnatally only, the incidence of animals bearing lymphomas/leukemias increased from 18.7% to 31.4%. The 2,000 ppm level corresponds to an assumed daily intake of 100 mg/kg body weight—approximately the equivalent of a 45-pound child drinking 5 cans of diet soda or a 150-pound adult consuming 14 packets of sweetener per day.

Although recent epidemiologic studies have not found an association between aspartame and human cancers, those studies were not designed to measure cancer risks associated with fetal exposures. The public health implications of the new findings are considerable. Currently, more than 200 million people regularly consume aspartame, and children and women of childbearing age (which presumably includes many who are pregnant and breastfeeding) are among the major consumers. If the U.S. FDA were to conclude that exposure to aspartame causes cancer in rodents, the agency would be required by law to revoke its approval for the popular sweetener. —**M. Nathaniel Mead**

The Best Rodent for the Job

NTP Workshop Compares Models

Researchers have long used rats and mice to understand the potential links between environmental exposures and incidences of breast, ovarian, testicular, and prostate tumors. Concern exists, however, that rodent models are not optimal for detecting carcinogens that act through the hormone system. To address this concern, the National Toxicology Program (NTP) organized a workshop in May 2006 to evaluate the utility of current two-year rodent bioassays to adequately evaluate these hormonally mediated tumors and the relevance of the findings to humans [*EHP* 115:1351–1356; Thayer and Foster].

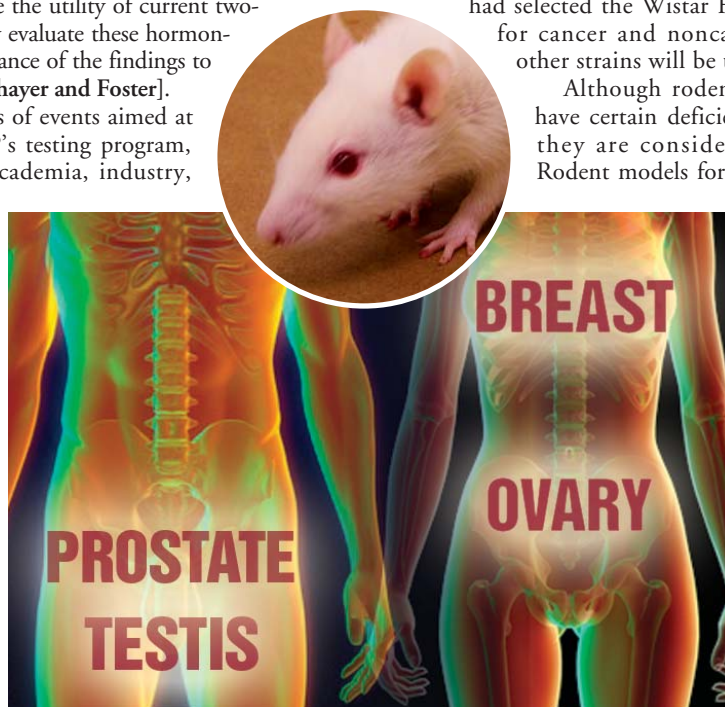
The workshop, part of a series of events aimed at evaluating and refining the NTP's testing program, included representatives from academia, industry, government, and nonprofit groups, as well as a panel of invited experts in endocrinology, cancer biology, reproductive toxicology, statistics, and other fields. Workshop participants were concerned that many hormonally mediated tumors, such as those in the testes and breasts, are initiated in fetal or early neonatal life, yet these periods of exposure are not covered in the NTP's standard cancer bioassay. In response to this concern, the NTP has committed to routinely include perinatal exposures in these studies unless there is a specific justification not to do so.

Furthermore, some rat and mouse strains used in testing either do not develop certain tumors or have a high incidence of spontaneous tumors. For example, the F344/N rat typically used by the NTP has high background incidences of testicular Leydig cell tumors and mononuclear cell leukemia, along with unresolved issues about declining fertility, sporadic seizures, and chylothorax (accumulation of lymphatic fluid in the pleural cavity, which can result from lymphoma). By press time, in

response to this and other workshop findings, the NTP had selected the Wistar Han as its standard rat strain for cancer and noncancer end points, although other strains will be used when appropriate.

Although rodent models are considered to have certain deficiencies and can be improved, they are considered valuable nonetheless. Rodent models for prostate and ovarian tumors

are the most problematic for understanding human disease because of significant interspecies differences in anatomy and tumor prevalence. Participants in the workshop recommended using alternative models, such as genetically engineered models and *in vitro* systems, to address some of these deficiencies. They also recommended more in-depth investigation of how noncancerous changes observed in rodents might be relevant to human diseases. —**Julia R. Barrett**



Baring Bone's Secrets

Understanding How Lead Exposure Affects Skeletal Development

Among lead's well-known developmental health effects is stunting of skeletal growth in children. Moreover, lead is known to delay fracture healing and may contribute to osteoporosis. Yet the exact mechanism by which lead affects normal cellular functions in bone and cartilage is poorly understood. A new study reports *in vitro* and *in vivo* effects of lead on cell signaling during differentiation of embryonic stem cells destined to form bone and cartilage [*EHP* 115:1276–1282; Zucsik et al].

Chondrogenesis is the process by which a mesenchymal cell—a type of stem cell already assigned to become connective tissue or blood cells—turns into a cartilage cell, or chondrocyte. As chondrocytes mature, they differentiate further into bone and more specialized types of cartilage. Because the early embryo makes a cartilage model of the skull, spine, and limbs, chondrogenesis is vital to full skeletal development.

The authors exposed mouse mesenchymal cells to lead *in vitro* and observed signaling changes in several proteins active in chondrogenesis: transforming growth factor-beta (TGF- β), bone morphogenetic protein (BMP), activating protein 1 (AP-1), and nuclear factor kappa B (NF κ B). They also implanted mesenchymal cells expressing BMP-2 in the thighs of living mice. Before implantation, the mice had been exposed to lead through their drinking water.

The *in vitro* experiment revealed several dose-dependent effects. Lead stimulated chondrogenesis, influenced the regulation of chondrogenesis by BMP-2 and TGF- β , and induced expression of three genes, also mediating the genes' regulation by BMP-2 and TGF- β . Similarly, in the *in vivo* experiment, lead was associated with a dose-dependent induction of chondrogenesis at the implantation site.

Lead also inhibited AP-1 signaling and induced NF κ B signaling. However, on the basis of previous research on AP-1 and NF κ B with opposite findings, the authors do not believe either of these pathways is responsible for the heightened chondrogenesis observed in the current study.

TGF- β proteins are mediated by members of the Smad family of transcription activators, and Smads also affect BMP signaling in some situations. In the current study, lead inhibited BMP-2 Smad signaling while stimulating the same in TGF- β . Lead's inhibition of BMP-2 Smad signaling "represents the most robust signaling effect identified to date in a skeletal cell type with regard to a candidate mechanism of [lead] toxicity," the authors state. At the same time, this finding implies that lead's influence on chondrogenesis operates independently of Smad signaling.

Given the importance of cartilage both in embryonic development and in fracture repair later in life, stimulation of chondrogenesis seems like a good thing; but if lead triggers the formation of too much cartilage at the wrong time, or prevents its further maturation into bone, this could explain lead's crippling effects on the skeleton. And because mesenchymal cells may differentiate into a variety of cell types in addition to cartilage, lead may also affect the development of other body systems.

—Valerie J. Brown



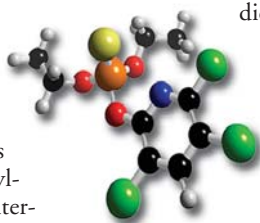
In Search of a Chlorpyrifos Antidote

Mechanisms Offer Clues

Treatments for acute organophosphate poisoning focus on stopping the buildup of the neurotransmitter acetylcholine at nerve endings. But organophosphates also interfere with neural cell development at much lower doses over a window of time that extends from fetal to neonatal development. This damage occurs through mechanisms that include direct interactions with acetylcholine receptors, interference with intracellular signaling cascades, and oxidative stress. Thus, any treatment aimed at stopping these sorts of effects would need to address those different mechanisms. Now researchers have advanced toward finding such an antidote [*EHP* 115:1306–1313; Slotkin et al.].

The team tested four treatments on the neurodevelopmental effects of the organophosphate pesticide chlorpyrifos. Using pheochromocytoma (PC12) cells, a tumor cell line that displays the major phases of neurodevelopment targeted by the pesticide, the authors evaluated the different treatments in terms of DNA synthesis, cell number (as indicated by measured amounts of DNA), cell size (as indicated by the ratio of protein to DNA), and cell signaling mediated by adenylyl cyclase.

Because chlorpyrifos can cause adverse effects by allowing acetylcholine to build up at nerve endings, the scientists tested two receptor agonists, atropine (which blocks muscarinic receptors) and mecamylamine (which blocks nicotinic receptors). This treatment



did not protect against the antimitotic action of chlorpyrifos: new cells failed to develop. However, once cells started to differentiate, the antagonists offered some protection against cell loss, although they could not prevent the deterioration of adenylyl cyclase signaling, which is essential for the development of neural networks.

The second treatment, nicotine, by itself had a small negative effect on the development of new cells, but it protected undifferentiated cells from the actions of chlorpyrifos and had mixed effects on cell numbers in differentiating cells. Nicotine both stimulates and blocks nicotinic receptors, and also possesses a mixture of pro- and antioxidant activity. The third treatment, the antioxidant vitamin E, also protected both undifferentiated and differentiating cells from many of the adverse effects of chlorpyrifos, but worsened the deterioration of adenylyl cyclase signaling. The fourth treatment, theophylline, a phosphodiesterase inhibitor that prevents the breakdown of cyclic adenosine monophosphate (the second messenger produced by adenylyl cyclase), was the only agent that restored cell signaling to normal or supranormal levels but did so at further cost to cell replication.

This new information indicates that it might be possible to design a cocktail of agents to counteract adverse neurodevelopmental effects of chlorpyrifos or other organophosphates, according to the authors. But they predict that finding an appropriate mix that avoids harmful effects inherent in the treatment agents, establishing doses, and determining whether the cocktail works for different chemicals will likely be a daunting task.

—Rebecca Renner