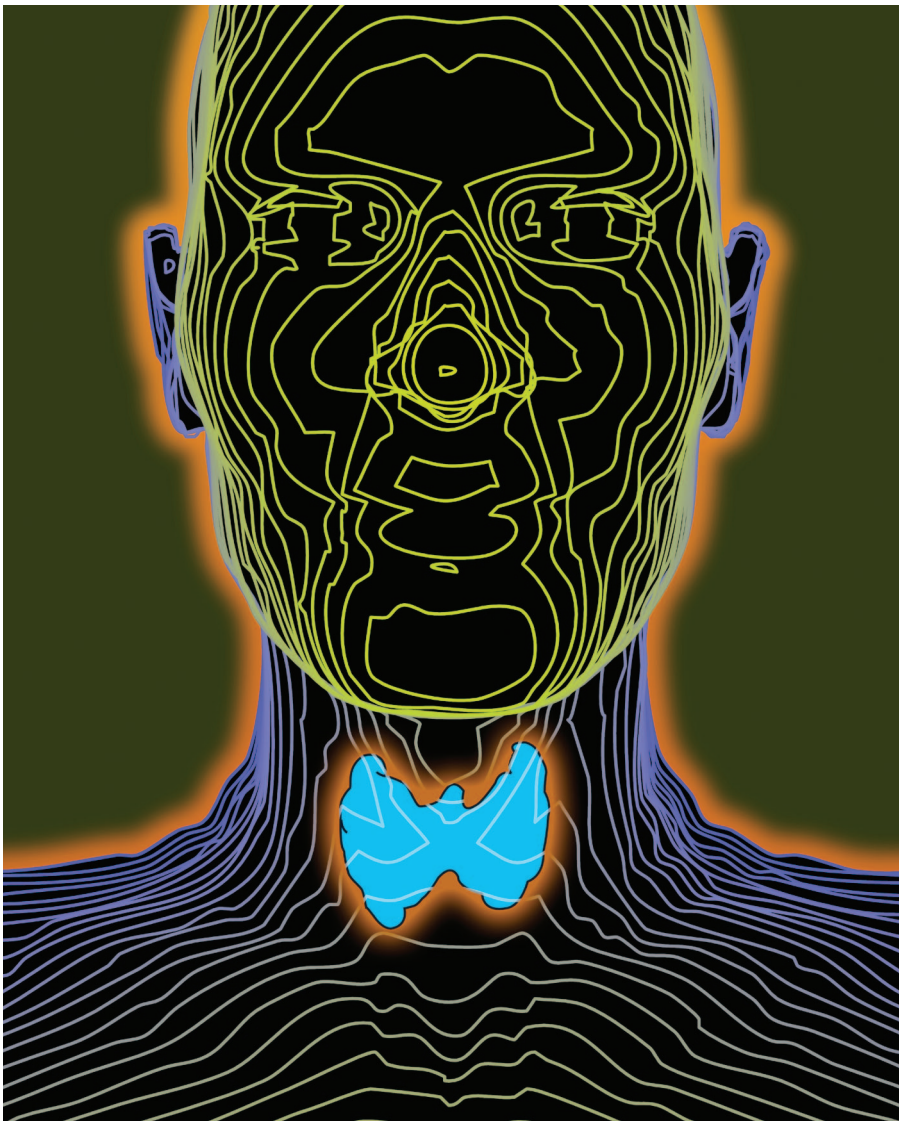
NIEHS-Funded Research Pursues Thyroid Findings

Clinical studies and animal experiments have established the essential role of thyroid hormone for normal brain development. A lack of thyroid hormone in pregnancy can result in congenital hypothyroidism, which causes moderate to severe mental retardation when untreated. Subtle thyroid hormone deficits in pregnant rats disrupt the migration of neurons in the fetal cortex and hippocampus. Several epidemiological studies have shown that mothers with subtle thyroid hormone deficiencies give birth to children with lower IQs than mothers with normal thyroid hormone levels. Exposure to certain environmental contaminants, including polychlorinated biphenyls (PCBs), organochlorine pesticides, and phthalates, has been reported to interfere with the production, transportation, and metabolism of thyroid hormone by a variety of mechanisms. Yet, despite the many puzzle pieces already in place, questions remain regarding whether—and to what extent—such effects by environmental contaminants include brain development problems.

The thyroid secretes thyroxine (T_4), which is converted in tissues to triiodothyronine (T_3); together, these two products generally are known collectively as "thyroid hormone." The activity of the thyroid gland is predominantly regulated by the pituitary glycoprotein hormone known as thyroid-stimulating hormone (TSH). Thyroid hormone exerts a negative feedback effect on pituitary secretion of TSH, so that when T_4 concentrations drop, TSH concentrations increase, thus keeping T_3 levels stable. Thyroid hormones exert their action at a nuclear level in the brain by regulating the transcription of thyroid hormone–responsive genes. This process is initiated when T_3 binds to thyroid hormone receptors. Perturbations in this system and the possible consequences are the topic of several NIEHS-funded studies.

Intramural and extramural research approaches range from animal studies of brain effects to studying U.S. populations that are exposed to relatively high levels of PCBs and other contaminants in comparison to the general population. In addition, a research group within NIEHS is exploring a novel mechanism by which thyroid hormone may affect brain development.



Big interest in a small organ. The NIEHS is currently funding a variety of studies examining the potential effects of environmentally related perturbations of thyroid function.

Brand X Pictures, Bryant Pate/EHP

PCBs: A Target of Research

A number of NIEHS-funded researchers are focusing on the effects of PCBs. PCBs are a well-studied class of industrial compounds that consist of paired phenyl rings with various degrees of chlorination. They are now ubiquitous and persistent environmental contaminants routinely found in samples of human and animal tissues. Although adverse effects have been linked to PCB exposure, it has not proved possible to tease out the mechanism of action for most of these effects.

Several studies have shown that exposure to this group of contaminants has been linked in humans with neurological effects similar to those associated with thyroid hormone deficiencies. In addition, there is strong evidence that exposure to PCBs can reduce circulating levels of thyroid hormone in animals (as reported by Ellen S. Goldey and Kevin M. Crofton in the September 1998 issue of *Toxicological Sciences*) and mimic thyroid hormone's effect on gene expression in the brain (as reported by R. Thomas Zoeller and colleagues in the January 2000 issue of *Endocrinology*). However, this evidence comes from studies using much larger PCB doses than those found in nature, says David Armstrong, a senior investigator in the NIEHS Membrane Signaling Group.

An association between PCB exposure and thyroid hormone levels in humans has been cited in multiple papers, including reports by Lars Hagmar and colleagues in the 10 April 2001 issue of *International Archives of Occupational and Environmental Health*, M. Sala and colleagues in the March 2001 issue of *Occupational & Environmental Medicine*, and Victoria Persky and colleagues in the December 2001 issue of *EHP*. PCBs can enhance liver metabolism of thyroid hormone, which

increases biliary excretion, or interfere with serum proteins that bind and transport thyroid hormone. Both effects may also reduce circulating levels of thyroid hormone.

Laboratory Studies

Zoeller, an endocrinologist at the University of Massachusetts Amherst, has, with his colleagues, identified a number of genes whose expression in the rat brain can be used as biomarkers of thyroid hormone action. Zoeller is particularly interested in the effect of thyroid hormone on early brain development, when the fetal brain gets thyroid hormone from the mother. By focusing on genes that are expressed before the onset of fetal thyroid function, Zoeller's group can study the effects of maternal exposure to thyroid-disruptive agents.

PCBs seem to mimic thyroid hormone's effects on expression of these genes—interfering with thyroid hormone signaling without necessarily inhibiting the function of the thyroid gland. However, Zoeller's group has yet to identify any PCB congeners that bind with the thyroid hormone receptor. Such binding is the most obvious mechanism for PCBs' mimicry of thyroid hormone action in the brain. Other contaminants that can competitively bind to thyroid hormone receptors include bisphenol A (as reported by Kenji Moriyama and colleagues in the November 2002 issue of the *Journal of Clinical Endocrinology & Metabolism*) and halogenated bisphenol A derivatives (as reported by Shigeyuki Kitamura and colleagues in the 26 April 2002 issue of *Biochemical and Biophysical Research Communications*).

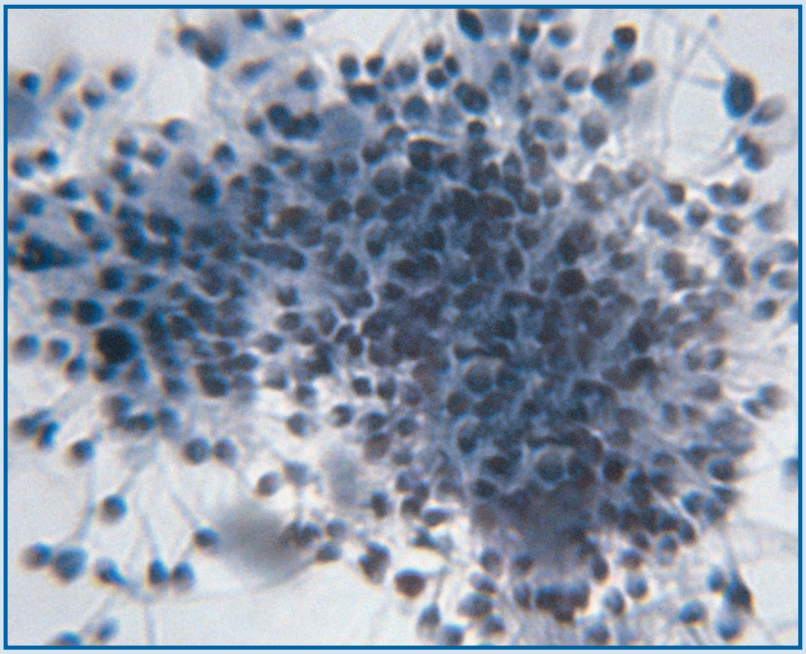
Lisa Opanashuk, an assistant professor of environmental medicine at the University of Rochester Medical Center in New York, has just begun working with *in vitro* cerebellar brain cell lines to look for indicators of contaminant action. While Zoeller started by studying the mechanisms of thyroid hormone action in the brain and then evaluating contaminant effects in this light, Opanashuk is first looking at contaminant action and then plans to eventually link these end points to thyroid hormone effects.

Armstrong and the NIEHS Membrane Signaling Group are taking yet another approach to studying thyroid hormone action in the brain by looking at basic mechanisms as opposed to toxicant action. Armstrong's team is pursuing tantalizing evidence that thyroid hormone receptors may also orchestrate nongenomic cellular functions in response to both thyroid hormone and environmental contaminants. In *in vitro* experiments using a rat pituitary cell line, Armstrong's team found that thyroid

Photodisc

Headliners | Male Infertility

NIEHS-Supported Research



Sperm Abnormalities in Men Exposed to PCBs and PCDFs

Hsu PC, Huang W, Yao WJ, Wu MH, Guo YL, Lambert GH. 2003. Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans. JAMA 289:2943–2944.

Polychlorinated biphenyls (PCBs) were once commonly used in transformers and other industrial applications because of their superior insulating properties and stability. When the adverse health effects of this class of compounds were discovered in the 1970s, they were banned in much of the world. However, because of their persistence, they are still among the most ubiquitous man-made environmental contaminants and are detectable in most human beings worldwide. Polychlorinated dibenzofurans (PCDFs), produced when PCBs are burned, are equally persistent and toxic.

During a six-month period in 1978–1979, approximately 2,000 people in Yucheng, Taiwan, consumed cooking oil contaminated with PCBs and PCDFs. PCBs had leaked from heat exchangers into the finished oil product. The PCBs were partially degraded by the heat, producing PCDFs and other chlorinated compounds. A registry of those exposed was created to track adverse health outcomes.

A previous study of prenatally exposed young men born to Yucheng women showed increased abnormal sperm morphology, reduced motility, and reduced fertility. In this research letter, NIEHS grantee George Lambert of the Robert Wood Johnson Medical School–University of Medicine and Dentistry of New Jersey in Piscataway and colleagues assessed the sperm quality of men directly exposed to PCBs and PCDFs in the Yucheng incident. Directly exposed men exhibited higher abnormal sperm morphology than controls and lower ability of sperm to penetrate hamster oocytes, a standard measure of fertility. Other semen characteristics were similar between exposed and control subjects. This is the first study to show adverse effects in sperm from men directly exposed to PCBs and PCDFs.

In the current study, the male-to-female offspring ratio was reduced in Yucheng men who were exposed before the age of 20. This suggests that the reduced capability of oocyte penetration found in this study may be specific to Y chromosome–bearing sperm, but this has not been confirmed and warrants further investigation. **–Jerry Phelps**

U.S.–Korean Toxicology Partnership Launched

As part of an initiative to build close working ties with international toxicology authorities, the National Toxicology Program (NTP) signed an exchange of letters with Korea's counterpart agency, also called the NTP, on 16 June 2003. The main goals of the partnership, which was spearheaded by U.S. NTP associate director Christopher Portier, are to help the Korean NTP build expertise in toxicologic methods and tao exchange data.

Other proposed priorities for the partnership include developing carcinogenicity assays, jointly conducting public reviews of technical data, and training Korean scientists in four areas: basic mechanisms of carcinogenesis, carcinogenic agents and the risk factors associated with them, genetic susceptibility, and statistical methods for data analysis.

Ties forged. Ki-Hwa Yang (left) and Kenneth Olden (right) partner their agencies as research allies.

The Korean NTP was formed last year within that nation's National Institute of Toxicological Research. The 15-year-old institute is part of the Korean Food and Drug Administration.

Korean NTP director Ki-Hwa Yang says the background of the program's 51 scientists is mainly in pharmacology and veterinary science. Korean scientists are eager to strengthen their knowledge of toxicological and testing protocols, he says, and are looking to model their program on the U.S. NTP blueprint and to incorporate the U.S. style into the technical reports they are planning to produce. A Korean toxicologist is already at the NIEHS for a three-month observation of the workings of the NIEHS/NTP, and plans are under way to bring over a Korean chemist as well.

Yang says one of the first focuses of the collaborative effort will be the study of botanically based medicines. He says botanicals are widely used in Korea, and little scientific research on their chemical properties and interactions with other pharmaceuticals has been performed there to date. Similar efforts are already under way in the United States, including NTP-funded studies on ginkgo, echinacea, ginseng, kava kava, and the plant compounds pulegone and thujone.

Portier is working to build similar links with Japan and Australia. Chris Schonwalder, senior environmental health advisor to the director of the John E. Fogarty International Center, says of this initiative, "Any and all things that we can do to assist . . . countries in increasing their scientific base for environmental policy should be encouraged. Not only is this good foreign policy, but it also leads to better scientific collaboration and new knowledge and understanding of human health risks." **–Erin E. Dooley**

hormone stimulates potassium channel activity through a signal transduction cascade that involves PI3 kinase and Rac GTPase. Other investigators have already reported that both Rac and potassium channels are essential for normal neurite outgrowth in the developing brain. Hence, this discovery may provide a novel, nongenomic molecular explanation for how thyroid hormone system perturbations can affect brain development.

Epidemiological Studies

Studies that have attempted to relate PCB body burden to thyroid hormone levels have produced inconsistent results, as have studies aimed at linking PCB exposure to neurodevelopmental effects. As part of his investigations into the developmental effects of organic contaminants, NIEHS epidemiologist Matthew Longnecker is exploring the reasons for these discrepancies.

Currently it's very difficult to compare studies because they use different measures of PCB exposure. To rectify this first barrier, Longnecker, together with other PCB investigators, has developed a uniform way to compare exposure levels so that data from all studies can be considered together. This method, reported in the January 2003 issue of *EHP*, focuses on the median level of PCB 153 in maternal serum. Longnecker and colleagues found that exposure in most studies overlapped substantially but that exposure levels determined in a study in the Faroe Islands, where people eat an especially high-PCB diet, were 3–4 times higher than the rest.

Epidemiologist Anne Sweeney and colleagues at Texas A&M University's School of Rural Public Health recently began a long-term prospective study of Asian-American children potentially exposed to high levels of PCBs and methylmercury through eating contaminated fish from Wisconsin's polluted Fox River. Researchers are currently recruiting reproductive-age couples with the goal of following 250 babies and parents from preconception through the children's growth.

Sweeney and colleagues will monitor changes in serum PCB and methylmercury concentrations and look for associations with perturbations in thyroid hormone status. The focus will be on specific aspects of cognitive, motor, and sensory function that are affected by PCBs and/or methylmercury in animal models and attributed to thyroid hormone disruption, but that have not been adequately assessed in human infants or children. These include auditory function, frontal lobe functions (such as working memory, planning, and response inhibition), and cerebellar functions (such as balance and coordination).

Lawrence Schell, an epidemiologist at the University at Albany School of Public Health, and colleagues are nearing completion of another population study to determine the effect of PCB exposure on thyroid hormone function in adolescents of the Akwesasne Mohawk Nation. Schell's team interviewed 270 mother–adolescent child pairs, collecting a diverse data set including information on maternal fish consumption; child diet; sociodemographic data; height, weight, and body composition measures; sexual maturation scale; and alcohol and cigarette use. Assessments of adolescent cognitive and behavioral characteristics were also conducted. Levels of total and free T_4, T_3, and TSH were then measured in the adolescents' blood.

Preliminary analysis of data from 117 adolescents in this study has found that the level of highly chlorinated congeners is significantly related to thyroid function, although it is important to note that the Akwesasne have been exposed to a large

Steve McCaw/Image Associates

Olden to Relinquish Directorships

On 29 July 2003, Kenneth Olden announced he will step down as director of the NIEHS and the National Toxicology Program (NTP) after 12 years of service in both capacities. Olden said his decision was based on his desire to spend more time with his family and to be more directly involved in the work of his research program in the NIEHS Laboratory of Molecular Carcinogenesis, which has continued since he became director of the NIEHS/NTP. Olden said he would remain until both posts could be filled.

Department of Health and Human Services secretary Tommy Thompson commended Olden on his vision, outreach, and communication efforts during his directorship, stating, "He has been an articulate and compelling spokesperson on the need for better scientific information for making important public policy decisions." Olden's efforts in these areas have included conducting Town Meetings around the country to gain input on decisions regarding NIEHS research activities and his support of the NIEHS journal *EHP*.

NIH director Elias Zerhouni noted that Olden has contributed greatly to biomedical research throughout his tenure as NIEHS/NTP director and has been a notably innovative leader, as evidenced by his work to bring minority scientists into the NIH program, to focus attention on health disparities, and to expand the scope of NIEHS research. From its core in basic biology, NIEHS research initiatives have grown to include studies such as the 50,000-woman Sister Study being conducted on breast cancer and to incorporate cutting-edge toxicogenomics tools in the search to determine how exposures to environmental chemicals affect human health.

number of harmful contaminants through living on the St. Lawrence River adjacent to a number of hazardous waste sites. The researchers emphasize that because the PCB levels in the sample are very low, their results may pertain to many populations with low exposure. According to Schell, these results support the theory that PCB exposure at levels commonly found in human populations alters thyroid function.

Other Efforts

An important part of both Sweeney's and Schell's population studies is an intensive effort to inform and involve communities in the research. This effort to involve the public is an important part of the NIEHS approach, according to Jerrold Heindel, a program administrator in the NIEHS Division of Extramural Research and Training. Researchers try to involve the community by explaining their work and requesting comments and input from the people involved. In some cases, such as the Fox River study, community outreach also involves showing people how to avoid PCB exposure, for example by avoiding certain fish and eating only skinned fillets.

The National Toxicology Program Center for the Evaluation of Risks to Human Reproduction is interested in developing tests to detect adverse reproductive and developmental effects resulting from chemical-induced thyroid dysfunction, says center director Michael Shelby. Such tests could be used to screen chemicals for thyroid hormone effects. Developmental toxicity tests do not routinely monitor thyroid status because there is no definitive, easily measurable biological end point whose consequences are well understood, he says.

Last September, the NIEHS cosponsored an international conference on thyroid hormone and brain development. Meeting participants agreed that thyroid hormone action plays multiple roles in both early and late brain development, and biomarkers to detect subtle effects are elusive. But new studies, conceived to pinpoint these effects by using genetically modified animals, are expected to make great strides soon. Together, all of these advances mean that there soon may be screening tests developed to determine whether environmental contaminants affect thyroid hormone signaling in the developing brain. Such knowledge could lead to a reduction in associated learning deficiencies. **–Rebecca Renner**

Steve McCaw/Image Associates