NIEHS News

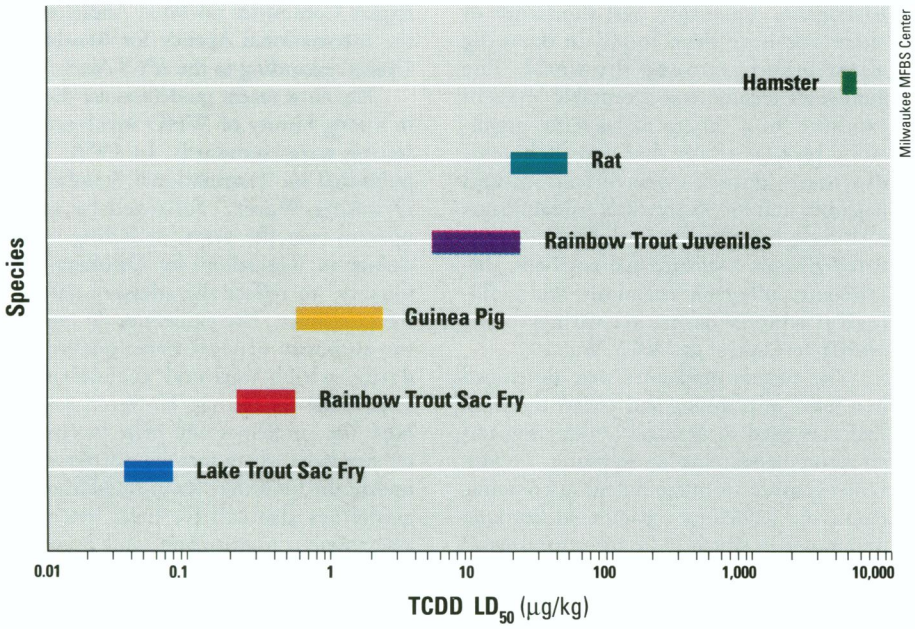
Fishing for Answers

Editor's note: This article on the Freshwater Biomedical Science Center at the University of Wisconsin at Milwaukee is the second in a series that appears intermittently in NIEHS News. The series highlights the activities of Environmental Health Sciences and Marine and Freshwater Biomedical Sciences Centers. The first article in the series appeared in volume 101, number 7.

In a cartoon in the *Far Side* series, an alligator is on the witness stand under interrogation by a human prosecutor. The alligator reacts angrily to a question with the retort, "Of course I killed him in cold blood, you fool; I'm a reptile." What seemed to be a legitimate question to the hot-blooded attorney was simply a fact of life for the belligerent creature. Why are we hilariously entertained by Gary Larson's odd cast of characters from all corners of the plant and animal kingdoms? Because they gently reveal so much about us as human beings. Whether to understand our individual and social behavior or to dissect our physiology and its diseases, humans have always learned by examining related mammals and other species, which act as surrogates in studies that, for ethical reasons, cannot be done with human subjects.

Fifteen years ago, there were many NIH centers devoted to animal research with mammals such as mice, rabbits, dogs, and primates to gain insights into the biological workings of humans. At that time, David Rall, director of NIEHS, inaugurated a bold program to support several centers that would use aquatic organisms to further our understanding of human environmental health problems. The Marine and Freshwater Biomedical Sciences (MFBS) Center in Milwaukee was among the first to be funded. Although alligators have yet to be used at the center, many other aquatic species have helped investigators in their research, such as a variety of freshwater fishes, several kinds of frogs, saltwater barnacles, and lobsters.

What did Rall recognize about aquatic organisms that convinced him to support centers devoted to investigating organisms that were a bit on the "far side" of traditional animal biomedical research? No doubt he recalled the importance of the squid in providing enormous neurons that made it possible to understand the functioning of nerve cells. He may have thought about the role that flounder played in defining how the human kidney works simply because this fish gave experimental access to a major part of our kidney structure. Whatever the case, these examples have multiplied over the years in each of the five aquatic centers supported by NIEHS.



Sensitive species. Studies of lake trout give clues to the species variability of dioxin toxicity.

Biomedical Research

The MFBS Center is located in the harbor of Milwaukee at the University of Wisconsin-System Great Lakes Research Facility. Funded through the University of Wisconsin-Milwaukee and directed by David Petering, a professor of chemistry, the center is a core facility that supports the activities of scientists from the University of Wisconsin-Milwaukee and -Madison, the Medical College of Wisconsin, and other regional institutions. Many of these scientists use mammals in their research and have been drawn to study aquatic organisms because they offer unique opportunities to advance their work. Like the squid, these aquatic organisms may present the scientist with novel physiological or anatomical characteristics that make possible experiments which are not possible with mammals. Aquatic organisms may provide practical economies of scale and effort that make experiments feasible, or these organisms may be directly affected by the environmental health issue of interest.

Richard Peterson, professor of pharmacy at the University of Wisconsin-Madison, works with fish for the first two reasons described above. He is known worldwide for his investigations of the toxicology of polychlorinated biphenyls (PCBs) in mammals. PCBs continue to present difficult questions for researchers trying to assess their possible hazard to humans. Laboratory rodents are relatively insensitive to PCBs, which presents the question of whether PCBs adversely affect humans. Recognizing that there is a huge range of toxic concentrations for PCBs among various species, Peterson has in recent years extended his efforts to examine how these chemicals affect the development of a highly sensitive species, lake trout, in order to understand this variability. Peterson's studies have been greatly facilitated by features common to many aquatic systems: large numbers of fertilized eggs can easily be maintained as they develop into juvenile and then adult fish; organisms at any stage of development can be uniformly exposed to toxic compounds in the water; and eggs can be treated directly with the toxic agent by microinjection.

Like Peterson, Janis Eells, a member of the Department of Pharmacology and Toxicology of the Medical College of Wisconsin, uses fish in her research

because they react differently to certain toxic compounds than rodents. Eells studies the neurotoxicology of pyrethroid insecticides, which account for about 30% of all the insecticides used in the United States. A thorough understanding of the molecular basis of the neurotoxicity of these compounds should help in designing a new generation of compounds that exhibit higher selectivity for insects and less toxicity to humans and other organisms in the environment. Fish such as the rainbow trout are much more sensitive to pyrethroid insecticides than are mammals. By comparing the underlying interactions of pyrethroids in trout and rats, Eells is beginning to define significant, differential effects and sites of action that may explain why these insecticides are particularly toxic to some species and not to others.

University of Wisconsin-Milwaukee chemist Frank Shaw has used the unique features of an aquatic organism in a different way to examine the molecular interactions of cadmium in cells. Collaborating with David Petering, Shaw is investigating the chemistry underlying the capacity of a protein, metallothionein, to protect cells from exposure to cadmium. Although related, lobster metallothionein differs in protein structure and metal-binding capacity from its mammalian counterpart. By comparing the structure and reactivity of the lobster protein with what is known about mammalian metallothionein, it is possible to relate differences in structure to gradations in function.

The difficulty facing most scientists who want to incorporate aquatic animals into their research is that many research institutions do not have the specialized facilities needed to support large numbers and types of aquatic species. For this reason the MFBS Center has become critical for researchers in Milwaukee. With its remodeling finished in 1991, the center now offers a state-of-the-art aquatic animal facility. The facility is overseen by Kris Kosteretz, an expert in both aquatic and animal husbandry and in experimentation with numerous aquatic organisms. Researchers can set up individualized experiments in isolated rooms, designed like mammalian biomedical animal quarters that provide total environmental control of water temperature, light, and sound. Specialized surgical procedures with toxic substances can be carried out, and a variety of assay systems of physiological function are available to center members. One such assay is the swimming tunnel, an aquatic equivalent of the treadmill, which is equipped with an oxygen monitor and port for a catheter, permitting the investigator to sample biological fluids over time.

Neurotoxic and Immunotoxic Chemicals Studies

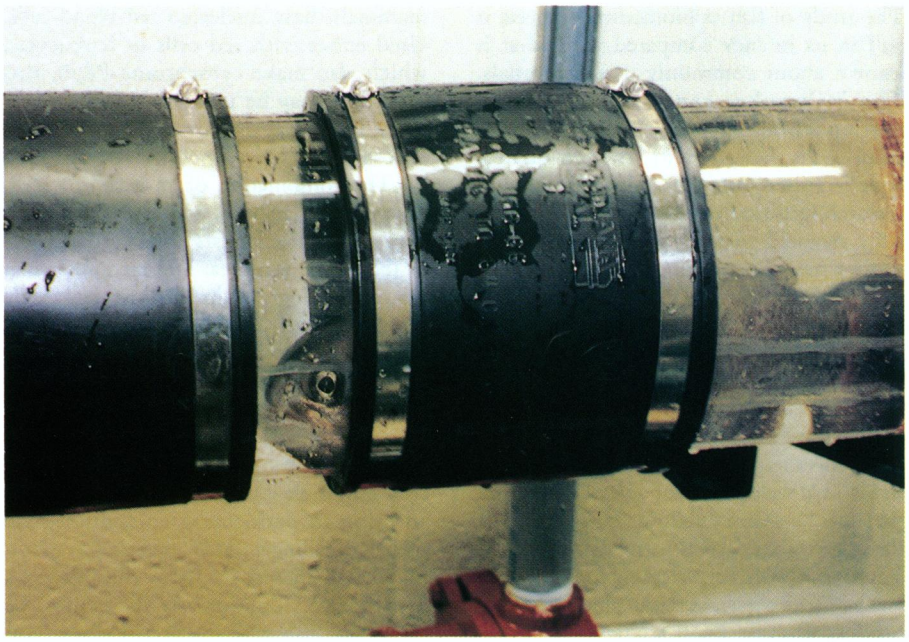
In addition to projects that focus on the effects of particular toxic agents on aquatic organisms, ongoing work explores the use of aquatic species in studies of physiological system toxicity. Leslie Zettergen, professor of biology at Carroll College, uses a method for simultaneously fertilizing large numbers of frog eggs and maintaining the resulting embryos to study the development of the amphibian immune system. He and others have shown that the frog and human cellular immune systems differentiate in similar ways, suggesting that this organism may provide an excellent model for studying and assaying human developmental immunotoxicity.

Daniel Weber, a staff member of the center, with Diane Seale, a member of the biological sciences department of University of Wisconsin-Milwaukee, and Nova University Professor Richard Spieler, formerly of the Milwaukee County Museum of Natural History, has found that fish such as fathead minnows and goldfish are remarkably sensitive to small concentrations of lead present in the water for relatively short periods compared with rodents, which are frequently used as models for childhood lead poisoning. At concentrations of lead that do not cause overt toxicity or anemia, the motor and learning capacities of these fish are subtly perturbed. Natural, individual, and social behaviors are altered, and underlying changes in the rhythms of neurotransmitters can be detected. Due to the ease of observing natural behaviors and actions of these fish in quasi-native aquarium environments, fish may provide a useful system for understanding neurobehavioral effects of low-level lead exposure in children. A recent report of the success in using goldfish in studies of chemically induced Parkinson's syndrome demonstrates that fish may offer new opportunities for studying neurobehavioral toxins.

Toxicological Studies

A significant amount of the protein consumed worldwide comes from fish and other aquatic creatures. In the United States, certain Native Americans get an even larger percentage of their protein by eating fish. In the Great Lakes area, in particular, sport fishing also elevates the amount of fish in the diet. The problem is that many fish are commonly contaminated by mercury, PCBs, and other organics that have either been dumped or transported through the air into bodies of water.

John Lech, professor of pharmacology and toxicology at the Medical College of Wisconsin, was the first director of the Milwaukee center and is a pioneer in the study of the uptake and biotransformation of chemicals in fish. One of his goals for many years has been to develop biomarkers for the presence of biologically active concentrations of toxic chemicals in fish. In cooperation with researcher Mary Haasch, Lech has developed field assay procedures to measure cytochrome P450 messenger RNA, which is a sensitive indication of the concentration of mRNA. mRNA is directly related to the concentration of cytochrome P450, the principal degradative enzyme for many organic chemicals. One study showed that the concentrations of this type of RNA are much higher in liver from fundulus, a type of fish, taken



Milwaukee MFBS Center

Against the tide. The center's swimming tunnel is the aquatic equivalent of a treadmill.

Milwaukee MFBS Center

Fish livers. P.C. Lee examines rainbow trout hepatocytes which will be used to study P450 induction.

from heavily polluted Newark Bay, New Jersey than from Tuckerton, New Jersey, a relatively clean area. Lech also measured cytochrome P450 protein activity, which varies directly with the extent of exposure of fish to certain toxic organic chemicals such as dioxin. The results of the study indicate that fish in Newark Bay are exposed to higher concentrations of these chemicals because the exposure was significant enough to induce the synthesis of this protective enzyme.

Specialized Facilities

The study of fish as biomedical subjects is still in its infancy compared with what is known about commonly used mammals. The Milwaukee center has sought to develop an on-site research staff and laboratories to complement its high-quality aquatic animal unit. In addition to typical research facilities, a cell culture room and a suite devoted to molecular biology techniques have been built at the remodeled center. Emphasis on both facilities and staff is critical if aquatic–based studies are to match the sophistication of studies using mammals.

P. C. Lee of the pediatrics department of the Medical College of Wisconsin has taken advantage of the cell culture equipment to develop a trout liver cell culture that can be maintained on minimal media for about a week without degradation of function. With this system, he and Lech have shifted their chemical biotransformation/cytochrome P450 studies to cell culture, where refined, controlled experiments of P450 induction can be done.

Lee, Lech, and Ruth Phillips, a well-known molecular biologist in the area of fish genetics from University of Wisconsin-Milwaukee, have also used the molecular biology facility extensively. Phillips and staff member Barbara Wimpee have devised a method to examine the integrity of genes in fish using only one microliter of fish blood. The technique is based on the power of the polymerase chain reaction to magnify the quantity of DNA in a sample. It also depends on the fact that fish, like most animals except mammals, have nucleated red blood cells. Used either with red cells or leukocytes, which also make cytochrome P450, this procedure may be useful to noninvasively assay DNA damage caused by chemical exposure.

The combination of an excellent staff, high-quality facilities, and an interdisciplinary group of scientists attracts researchers to the Wisconsin area. For example, when neurotoxicologist John Dellinger recently moved to the Medical College of Wisconsin, he immediately began to consider the possibilities of working at the center. His research focuses on the possible neurotoxicological effects of fish consumption on Native American populations, who eat fish containing elevated levels of mercury. In addition to performing epidemiological studies, Dellinger feeds mercury-contaminated fish to rats to assess the effect on neurological function. At the center, he can obtain fish with the precise, long-term exposure to mercurial compounds necessary to mimic mercury uptake in the wild.

The center regularly hosts workshops, symposia, and courses on the use of aquatic organisms in biomedical research. Starting this past summer, the center offers a month-long course on molecular techniques in aquatic biomedical research with particular emphasis on their application in toxicology. Topics include major methods in molecular biology, an introduction to ways that fish can be used in biomedical research, and applications in the field of toxicology described in seminars by members of the center. The course is taught by molecular biologists and Barbara Wimpee, Charles Wimpee, and Chun-Mean Lin from the University of Maryland Center for Marine Biotechnology. A former member of the center, Kevin Kleinow, a professor of aquatic veterinary medicine at Louisiana State University, gives demonstrations of biomedical techniques using fish in the research lab.

The Milwaukee Marine and Freshwater Biomedical Sciences Center is a unique resource for the Great Lakes area and comprises an unusual array of scientific interests. Environmental health studies using aquatic species naturally break down barriers and link a wide variety of scientific disciplines and interests.

Fine Particles in Air Shorten Lives

In a study published December 9 in the *New England Journal of Medicine*, Harvard investigators found that exposure to fine particle air pollution, such as that produced by soot, increased the risk of early death by 26%. In a comparison of individuals living in six communities in the United States, those in the most polluted cities had a 26% greater mortality rate than individuals living in the least polluted cities. The life expectancy of an individual in the most polluted cities is shortened by one to two years. Fine particulate air pollution, produced by industrial and automobile emissions, was the pollutant most strongly associated with increased mortality.

The Harvard Six-Cities Study is an ongoing examination of the health effects of air pollution that has been supported for 20 years through a grant from NIEHS, with supplementary support from the EPA Health Effects Research Laboratory and the Electric Power Research Institute.

"Perhaps most significant is that the particles measured in our study are much smaller than the particle diameter standard currently used by the U.S. Environmental Protection Agency," commented Douglas Dockery, associate professor of environmental epidemiology at the Harvard School of Public Health and lead author of the study. "We are particularly concerned