Pesticide exposure and the developing brain: Combining molecular, behavioral, field studies for more complete picture

Studying the effects of pesticide exposure on children is in some ways a matter of putting together a puzzle that up to now has been missing a few pieces. Scientists know that pesticides—designed to attack the nervous systems of bugs—can do the same to humans at high exposures, but they don't know how the lower, environmentally relevant levels of pesticides may affect a brain that is still developing. They know that children can be more susceptible to environmental toxicants than adults, but they don't know how much more sensitive they may be. They know that pesticide exposure has effects at the molecular level, but they don't know if these effects translate into altered behavioral development. And they know that children of agricultural workers have greater-than-expected levels of pesticide residues in their urine, but they don't know just how the exposure occurred or how dangerous it is.

The UW’s new Center for Child Environmental Health Risks Research is attempting to find some of the missing pieces and to insert them into the puzzle to make a more complete picture. “We have designed a center that links molecular studies with behavioral studies and both of these with field studies.”

Dr. Elaine Faustman, Pediatrics Professor M. Mirkes and Environmental Health Professor Faustman will be studying these processes during three “windows of sensitivity” in children’s neurodevelopment: in utero midbrain development and postnatal development of the cerebellum and hippocampus. The midbrain is known to be involved in the relaying of sensory-motor information between the cortex and spinal cord in vision and in hearing. The hippocampus is important in spatial learning and memory, while the cerebellum is critical for motor activity.

For the embryonic part we’re taking cells from the midbrain of rat embryos, dissociating them and culturing them in vitro where they continue to grow and

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devlop,” Mirkes says. “We’ll then be exposing these cells to doses of the pesticides. We’ll follow that up with exposures in vivo in the same stage from which these cells were obtained. In other words, in a pregnant rat.” A similar procedure will be followed for the cerebellum and the hippocampus, using newborn rats.

The question is, does exposure to pesticides prevent or slow cell proliferation? And does it cause unprogrammed cell death? Faustman will concentrate on the cell proliferation aspect, while Mirkes will study cell death, a subject he has been investigating for several years, though never in connection with pesticides.

“Cell death is a logical thing to study,” he says, “because many agents that are known to cause birth defects in animals start off by killing off cells in specific parts of the embryo.”

However, if the pesticides are shown to alter cell proliferation and cell death in the developing rat brain, it doesn’t prove that they cause deficits in functioning. That’s why the center also includes experiments testing how pesticide-exposed rats perform compared to non-exposed peers. Another CHDD research affiliate, Dr. Tom Burbacher, is in charge of that part of the work.

The study will begin with fairly gross measurements of newborn rats: birth weight, size, ability to right themselves. But the researchers don’t expect to find differences there. “You would not normally find effects on gross motor capabilities at birth unless you had high exposures,” says Burbacher, who is an associate professor of environmental health, “so most of what we’re going to do is geared to looking at more subtle kinds of effects.”

When the animals are weaned, at about 21 days, their activity levels will be tested in both short and long-term situations. One of the known indicators of problems with exposure is either hyperactivity or hypoactivity, so researchers will be looking for that.

At about 40 to 60 days, tests of learning and memory will begin. Rats will be evaluated on such tasks as learning a maze and discriminating between different kinds of stimuli. By the time these tests are done, results of the earlier, molecular studies will be available, so that if there are differences between exposed and non-exposed rats, it will be possible to make connections between cellular alterations at specific times and with specific doses and the rat’s level of functioning. One hypothesis of the study, in fact, is that “certain pesticides affect learning, growth and development by altering the balance between cell proliferation and cell death.”

The connection between cellular and developmental studies is one aspect of the center’s work that is new. Another is the emphasis on risk assessment. Up to now, regulatory agencies have assumed a tenfold difference between adults and children. In other words, if a substance is safe at x level for adults, it would be safe for children at only one-tenth of that. But the tenfold rule is only a guess. In fact, in the developing brain, the timing of the exposure can be as important as the amount. For example, classic studies with radiation exposures in
CHDD researchers' intervention program fosters social competence in young children with developmental delays

As young children play together they are refining skills they will use throughout their lives to interact with other people—they are developing social competence. Acquiring the skills necessary to build satisfying peer relationships can be a rough road to travel, but most young children learn to negotiate the bumps. Only about 10 percent of children who are otherwise typically developing have trouble making friends.

However, the problem is far more widespread among children with developmental (cognitive) delays, according to Dr. Michael Guralnick, professor of psychology and pediatrics. It is estimated that more than half of children with mild developmental delays have substantially poorer peer-related social competence than would be expected given their individual developmental levels. A child's social competence with peers is characterized by the appropriateness of strategies he or she employs and whether he or she is successful in accomplishing specific social tasks. Social tasks include the ability to join in to a group of children at play, maintain play with peers and resolve conflicts.

Children with little social competence have trouble developing friendships and are frequently isolated. They often miss the process of testing ideas out with other children and getting the feedback they need to learn a variety of important cognitive-social and communicative skills. "It's not only a problem in terms of developing relationships, it affects other areas of development as well," says Guralnick.

Guralnick, director and a research affiliate of CHDD, has investigated factors involved in peer-related social competence for more than 20 years. He and his colleagues have conceptually modeled socially competent behavior in young children with mild developmental delays. Based on that model, they have designed an assessment and early intervention program to improve peer-related social competence in these children. The researchers are evaluating this intervention in a four-year, $1.1 million project funded by the National Institute of Child Health and Human Development (NICHD).

At least 100 children with mild developmental delays between 4 and 6 years of age and their families will be enrolled in this clinical trial. The comprehensive intervention is a coordinated school- and home-based program, which is individualized based on assessments of the child's functional level in the processes that underlie social competence.

"In developing the model of factors that govern socially competent behavior we've identified an array of social-cognitive and emotional regulation processes that are important underlying features of social competence," explains Guralnick. A child with developmental delay may have trouble with some of these processes due to his or her general difficulties in processing information or remaining focused on a task.

However, there is an additional aspect to consider, says Guralnick. "Although some of the underlying processes might not operate effectively due to intrinsic child characteristics, there are also many family factors that we now know contribute to the development of these processes. Family-child relationships, experiences with other children that are organized by families, the kinds of play that parents have with their children—all are associated with these different processes."

The home-based intervention component involves working with families, primarily mothers, to help them interact more effectively with their children during play situations, to help them regulate their emotions during play and to help families organize social experiences with peers for their children. "Many children with developmental delays don't have access to other children as readily as do typically developing children, which further limits their experiences," notes Guralnick. "We provide suggestions about how to enhance the experiences they do have playing with other children, and we work with the mothers to figure out how to support that play so that it goes well.

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Glimpsing your own future in DNA: Researchers seek to learn who chooses knowledge and what impact it has

Suppose a genetic disease runs in your family, a disease in which symptoms frequently don’t appear until adulthood. Suppose you’re 21 and symptom-free. Would you want to know if you carried the gene that would eventually trigger the disease?

As recently as 10 years ago, that question was moot for many diseases, because the telltale genes hadn’t been identified and therefore there was no way to know who did and didn’t carry them. But as scientists unravel the genetic code, discovering specifically which genetic aberrations cause particular conditions, what follows soon after are DNA tests that can determine—often through a simple blood draw—whether a given individual does or does not carry a flawed gene. Suddenly, symptom-free people have the opportunity to get a glimpse into their own future. Will they take it, and if so, how will the knowledge affect their lives?

These are the questions CHDD researchers are trying to answer in a preliminary study of four common hereditary diseases of the nervous system: Charcot-Marie-Tooth disease, myotonic muscular dystrophy, facio-scalpulo-humeral dystrophy (FSH) and the hereditary ataxias.

“What little is known about how DNA testing actually affects people, both short term and long term,” says CHDD research affiliate Dr. Tom Bird, who is directing the study. “That’s why we’re doing this—to get some concrete data.”

Much of the data about genetic testing that does exist comes from another disease of the nervous system, Huntington’s disease (HD). The gene for HD was discovered in 1993; the DNA test became available about a year later. Prior to its availability, 75 percent of those at risk said they would take the test, but the actual participation rate has been much lower. Only about 25 percent of those at risk have chosen to be tested.

That’s not too surprising, says Bird, who is a professor of neurology and also of medicine and medical genetics. “It’s human nature for people to say they’d do something when it’s only theoretical, then back off when it becomes a reality.”

But Bird doesn’t think that Huntington’s disease is a good model for all genetic diseases or even for all genetic diseases of the nervous system. The problem with HD as a model, he says, is its severity. The disease always affects the brain, interfering with cognitive functions; it causes involuntary body movements; and it shortens the life span. Although the diseases he is studying are all incurable, they vary in severity level and none has all three of these characteristics. Individuals at risk may therefore see them as less threatening—something they could more easily cope with, and this may in turn affect their attitude toward and reaction to genetic testing.

Nevertheless, although the staff of the UW’s Medical Genetics Clinic have told affected families about the study, at risk individuals “haven’t exactly been breaking down our doors to enroll,” Bird says. The group plans additional recruiting efforts to attract more study subjects, but he adds, “I suspect we won’t be able to get enough people to generate powerful statistics, so our study is likely to be descriptive.”

What Bird and his assistants, genetic counselor Corrie Smith and research nurse Hillary Lipe, plan to do is to ask at risk individuals to fill out questionnaires before and after DNA testing. Then, they’ll be re-contacted yearly through the five-year span of the study to see how they’re feeling and how their lives have been affected, regardless of whether the tests were positive or negative.

Two of the questionnaires to be administered are standard measures of depression and anxiety. A third is tailored to the study and asks such questions as how the individual views the disease, what issues he or she considers important in considering DNA testing and who will be told about the test results. After testing, this tailored questionnaire asks how the testing has affected the individual in areas such as relationships, family planning and employment. Researchers plan to use the

“There’s a lot of societal worry about genetic testing, but very little data. We’re really excited that we’ll be able to generate some concrete information about how genetic testing impacts the lives of people.”

Dr. Tom Bird

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questionnaires to analyze what factors influence the decision to have or not have DNA testing, what the immediate effects are and what the long-term effects are. They also want to compare results for the different diseases under study.

Similar studies that have been done on people who were at risk for HD have found that most of those who tested positive are coping well, Bird says. There have been few suicides, which was the first concern, and even depression seems to be confined to a small subset of people. Surprisingly, some of those who learned they didn’t have the HD gene also had problems. Some had “survivor guilt” — feeling bad that they had escaped when others in the family had not — and others were disappointed when their lives didn’t suddenly get better after they learned they didn’t have the disease.

No systematic studies have as yet looked at practical matters such as the effect of DNA testing on employment and insurance. “People have a lot of fear that if they test positive for a disease, they may have trouble getting a job or getting insurance,” Bird says. “This may be one reason why people are not eager to have the tests done.” But he says that although there is some anecdotal evidence of people having such difficulty, it doesn’t appear to be a widespread problem—at least not yet.

In fact, one of the results of studies such as this one will be information that can be used by regulatory agencies, the government and others in developing policies, rules and regulations. “DNA testing is so new that no one really has a strategy for dealing with it yet,” Bird says.

The researchers also hope the results will be helpful to them in counseling people who are considering DNA testing. In sharing their concerns on the surveys, participants will be telling those who do the counseling just what should be discussed. And the researchers plan to take the extra step of writing informational booklets for each of the four diseases under study. Corrie Smith will be the primary author of those booklets.

“One of the needs in this field is for education and information for people who have these diseases and for their families who are at risk for them,” Bird says. “There hasn’t been a lot written for the lay public about DNA testing. One of our genetic counselors, Robin Bennett, wrote a beautiful booklet focusing on HD that we’ve been using for several years. So our plan is to create a similar booklet for each of these diseases that we can give to people who are considering DNA testing.”

The researchers have just completed the first year of the five-year study, which is funded by the National Institute for Disability and Rehabilitation Research of the Department of Education. They hope the results will shed some light in an area where there has been little beyond speculation. “There’s a lot of societal worry about genetic testing, but very little data,” Bird says. “We’re really excited that we’ll be able to generate some concrete information about how genetic testing impacts the lives of people.”

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Social competence development

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“We have specific intervention tools—protocols derived from the assessments—that are provided to both parents and teachers. These protocols provide a blueprint for the kinds of supports that parents and teachers need to provide the child to promote peer-related social competence. The protocols we choose depend on the particular child’s assessment.”

To evaluate the intervention’s effectiveness Guralnick and his colleagues on the project, Drs. Mary Hammond, Robert Connor and Brian Neville, are using data gathered during laboratory observations of children in the study both before and after the intervention program. Three different play situations are set up in the laboratory, where the researchers can maintain control over the circumstances and thus compare social skills across children. Each child in the study is observed in play with a friend or otherwise familiar child, with his or her mother and with a small playgroup of children with whom the child is unfamiliar.

The play sessions are videotaped for analysis by coders who watch for specific behaviors during the stream of play that can be categorized and recorded. Both of the child-peer play situations are coded with two different coding systems. One system indicates how connected the child is to other children in play and the other focuses in on what behaviors might be associated with the child’s successes and failures in play.

The child-parent play includes a free-play session and a session devoted to duplicating a toy model. The audio portion of the entire free-play session is transcribed to analyze the interactions in terms of turns. “By focusing on the communicative exchanges we can get the flavor of how the play is put together,” says Neville. “We take a microscopic look within each turn by coding for statements, requests and directives. We then lump them together and code each turn for its relevance to the previous turn. This allows us to capture how long the child and parent stay engaged in an activity. Length of engagement during parent-child play has been predictive in other studies of peer-related social competence.”

The model-building session, which is set up as a series of increasingly difficult tasks, is coded for the mother’s attempts to provide the minimal but necessary support the child needs to complete a task. “This allows us to know if the parent is providing too much or too little support,” explains Neville. “Social tasks also have varying levels of complexity so this information enables us to help parents figure out how much support the kids need in order to be successful during play.”

The project’s assessments and related intervention protocols are designed to become tools to be used by community-based specialists who work with children with disabilities. One of the assessments, The Assessment of Peer Relations (APR) developed by Guralnick in 1992, is currently in use in a number of school settings. “With this project we’re formalizing the intervention protocols and their linkages to the APR,” says Guralnick. “We’ve also developed a family version of the APR that focuses strictly on the children’s social skills along with a broader program for families that includes assessments of stress, attitudes, and how children and families interact with one another.”

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Pesticide study

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The rat have shown that doubling the dose (from 50 to 100 Rads) on day 9 of rodent gestation can cause a greater than fourfold increase in rat brain malformations (9 percent to 41 percent incidence). On day 10, one day later, exposure to 50 Rads does not produce any brain malformation and 100 Rads produces only 8 percent incidence.

Faustman, one of whose research specialties is risk assessment, will be using data from both the molecular studies and the developmental studies to create a dose-response model for the pesticides under consideration. This should help federal regulatory agencies in setting protective exposure limits for children.

The center won’t be stopping there. Communities in Eastern Washington have agreed to participate in studies—to be directed by other UW investigators and some at Fred Hutchinson Cancer Research Center and the Washington State Migrant Council—pinpointing how children are exposed to the pesticides and what the best preventive measures would be. Earlier work by environmental health faculty has shown that the children of farmworkers have residues of pesticides in their urine that are higher than children whose parents don’t work in the fields. The question is, how did the pesticides get to the children, who are not themselves in the fields? Once the answer is known, preventive measures can be taken. A risk communication study will be part of the work.

The UW center is one of eight nationwide funded by the Environmental Protection Agency and the National Institute of Environmental Health Sciences. Although all the centers focus on environmental toxicants and their effect on children, only two—including the UW’s—will be looking at pesticides. They’re particularly rewarding for a professor of public health to study, Faustman says, because the probability of preventing problems is high. “We do have some control over how and when and in what forms we apply pesticides,” she says. “That’s not always true with environmental toxicants. Our goal is always to be preventative.”

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Bringing health care providers, adults with developmental disabilities together is aim of new CHDD-DDC project

When a child shows signs of having a developmental disability, his or her parents can usually find appropriate health care for that child, including a pediatrician and many specialists. But when the child grows up, health care often becomes a problem, especially for people who are able to live relatively independently.

"What we hear from self advocates, parents and caregivers is that adults with developmental disabilities often have difficulty finding appropriate health care," says Donna Patrick, manager of public policy and advocacy programs at the Washington State Developmental Disabilities Council (DDC). "What we hear from health care providers is that they don't know very much about developmental disabilities, and that they're not comfortable dealing with some of the behavioral issues involved in interacting with adults who have these disabilities."

Acting on the assumption that at least some of the unmet service needs of adults with developmental disabilities are related to unmet training needs of health care providers, CHDD researchers have begun a study of the barriers adults with disabilities face in their search for health care and will design training for health care providers based on what they learn. The project is funded through a contract with the DDC.

"This is something that a lot of us in the field have wanted to do for a long time," says Dr. Douglas Cook, director of the Adults and Elders Program in CHDD's University Affiliated Program and co-PI of the project. "It was clear 20 years ago that we had a framework in the medical community that dealt well with children, but we have never had a framework that deals well with adults."

Part of the reason for that, of course, is that there was a time when few children with developmental disabilities survived into adulthood, and those that did either lived at home or were institutionalized. In the 1930s, for example, the average lifespan for a child with Down syndrome was five years; today that same child is likely to survive into middle age and beyond. And as people with developmental disabilities age, they face the same health risks as people without disabilities.

"The problem physicians have is how to maintain a balance between recognizing that a person's health problem may be related to his or her disability and yet not assuming that it is," says Dr. John McLaughlin, director of the UAP's Clinical Training Unit and co-PI of the project. "There are secondary health problems related to Down syndrome, for example, but a person with Down syndrome could also have unrelated health problems that shouldn't be overlooked."

Family members often complain about this, Cook says. A physician will attribute new health problems to the disability when family members, who are more familiar with the patient, knows it is something different. That leads to frustration on the part of the family and poor services for the individual with the disability.

Another common problem, according to McLaughlin, is that an adult with a developmental disability— who is more likely these days to be living independently— will see a physician who isn't aware of the disability. Because the vast majority of disabilities are on the mild end of the spectrum and not obvious, the physician will simply explain, for example, a medication in the usual way, thinking that the patient understands. The adult with a disability then ends up looking like a noncompliant patient because he doesn't understand the explanation and doesn't take the medication.

Because of these and other difficulties, many health care professionals have simply avoided treating people with developmental disabilities, Cook says. This has led to a situation in which many pediatricians are continuing to see patients with disabilities into their 20s and even their 30s. And there are many other adults with developmental disabilities who just aren't getting health care, particularly preventive health care.

"We know for example that women with disabilities don't get mammograms in anywhere near the numbers that non-disabled women do," Cook said. "And it's the same situation with men who have disabilities and prostate exams."

The contract that CHDD researchers have with the state DDC gives them $50,000 per year for up to three years with the idea that they will find out what kind of training health care providers need to work comfortably with adults who have developmental disabilities and then design that training.

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New research affiliate

Dr. David Haynor is an associate professor of radiology. He received a doctoral degree in mathematics from the University of California, Berkeley and a medical degree from Harvard Medical School. His research involves applications of image processing, such as morphometrics, functional MRI, brain perfusion and white matter tract mapping. He is particularly interested in using these techniques to track structural brain differences in fetal alcohol syndrome, disorders of development and neurodegenerative disease.

Social competence

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Guralnick and his colleagues plan to follow both intervention and control group children in the study for six years. At the end of the current project they will have a measure of the effectiveness of the intervention program and an elaborate set of measures pointing to factors that are the most powerful in producing change.

To be eligible for participating in the study, a child must be 4 to 6 years old with an Individualized Educational Program (IEP) and an inclusive placement within a school district in the Seattle metropolitan area. Families concerned about the social skills of a child who meets these criteria can call the Children’s Friendships and Families Study at 206-543-8750 for more information.

Adults and health care

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The first step in the process was a literature review, completed this summer. The review is rather short, Cook says, because very little research has addressed this issue. The state of California has recently begun a massive project mandated by the legislature in which they will be creating teaching modules for health care professionals. CHDD researchers hope to reap the benefits of the California work as it moves forward. In the meantime, they’re sending out survey forms to families, caregivers and health care professionals trying to find out what their experience has been and what some of the barriers to good health care for the developmentally disabled population are. The adults with disabilities themselves will participate in group interviews at a later time.

Because a mailing list was readily available, researchers are starting with occupational therapists among the health care providers, but Cook also had plans to hand out survey forms at the Washington State Medical Association’s meeting this fall. Although the survey forms are anonymous, they are marked as to which group a respondent belongs to and also the geographic area in which he or she lives. “The DDC is concerned about whether there is a more severe problem in rural areas and wanted us to look at that aspect of it,” Cook says.

When the survey forms are in and analyzed, CHDD researchers will then move to designing training materials. “Health care professionals need two things,” Cook says. “They need more knowledge about the various kinds of developmental disabilities, and they need some guidance on how to interact with someone who has a disability.”

Few health professionals routinely get such training, and most of what is offered is brief. Cook is not sure at this point what will be included in the new training, or even whether it will be offered in face-to-face or media format. There are arguments on both sides, he says. But whatever form training takes, Cook is excited that CHDD is leading the drive to address a long-term need.

The DDC is also pleased. “We have great hopes for the project,” Patrick says. “We think if health care professionals can be made to feel comfortable dealing with adults who have developmental disabilities, and that if we provide them with ongoing resources, that population is bound to be better served.”