Understanding Sex Differences in Environmental Health: A Thought Leaders' Roundtable

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Under the auspices of the Society for Women's Health Research, a thought leaders' roundtable was convened at the National Institute of Environmental Health Sciences in October 2002 to discuss recent advances in environmental health research, particularly those findings that explain sex differences in response to environmental exposures. Researchers discussed the latest findings on the interaction between sex and environmental exposures on health. Participants concluded that a greater focus on interdisciplinary, hypothesis-driven research is essential to advancing the field. To understand fully the potential effect of chronic exposures, researchers need to develop models to explore not only physiologic sex differences but also behavioral responses to low-dose and multiple chemical exposures. Future research should examine sex differences from the cell line to behaviors and should track these differences across multiple generations. Federal agencies should support such research in their awards of investigator-initiated grants. *Key words:* **autoimmunity, endocrine disruptors, heavy metals, immunology, sex differences, steroid receptors.** *Environ Health Perspect* **112:604–609 (2004). doi:10.1289/ehp.6714 available via** *http://dx.doi.org/* **[Online 18 December 2003]**

More than 80,000 chemicals are registered for use in commerce in the United States, and an estimated 2,000 new ones are introduced annually. These chemicals are used in everyday items such as foods, personal care products, prescription drugs, household cleaners, and lawn care products [National Toxicology Program (NTP) 2002]. Although environmental health research has made great strides in comprehending the impact of these chemicals on human health, the sheer volume of new chemicals being introduced daily complicates our understanding of the compound effects of multiple environmental exposures on health over time.

In October 2002 the Society for Women's Health Research convened a thought leaders' roundtable at the National Institute of Environmental Health Sciences (NIEHS; Research Triangle Park, NC) to discuss recent advances in environmental health research, particularly those findings that explain sex differences in response to environmental exposures. The environment plays a crucial role in the etiology of many diseases, including cancers and disorders of the endocrine, nervous, and immune systems. Understanding how such agents disrupt biologic processes in both sexes will allow us not only to prevent and possibly cure many diseases and disorders but also to explain their disproportionate impact on men's and women's health.

Participants in this meeting presented research from a broad spectrum of disciplines ranging from immunology to behavior, using both animal and human models for disease. Their discussions, summarized in this article, highlight the need for an interdisciplinary approach to environmental health sciences. An interdisciplinary approach is particularly appropriate when addressing the questions that arise when sex differences are looked for and ultimately found. The study of sex differences is emerging as an important area of biomedical research, and this workshop illustrates the complexities inherent in such studies, which span endocrinology, molecular genetics, microbiology, development, behavior, and multiple generations.

The Environment and the Immune System

The impact of the environment on the immune system is well known. Malnutrition, for example, can lead to a decrease in lymphocytes, particularly affecting the immature $CD4^+$ $CD8^+$ cells, and to atrophy of lymphoid tissue (Pallaro et al. 2001; Savino 2002). Immunosuppressors such as cyclosporine and FK-506, steroids, chemotherapeutic agents, and other drugs can depress the immune system (Medscape DrugInfo 2003; Patel et al. 2003), and environmental exposure to a variety of toxic chemicals such as dioxins, PCBs, heavy metals, pesticides, herbicides, and particulates from burnt fossil fuel can reduce immune responses and increase sensitivity to infection (Selgrade et al. 1999). These diverse chemical and biologic agents may act in very different ways, and we have few clues regarding the differential effects of chemical combinations on the sexes and across generations.

Allen Silverstone, of the State University of New York Upstate Medical University (Syracuse, NY), showed that both corticosteroids and dioxin pollutants cause atrophy of the thymus through very different mechanisms of action. A single dose of dexamethasone causes thymus atrophy in laboratory mice within 48 hr, followed by a slow recovery over the next 2 weeks. However, transgenic mice expressing the human anti-apoptotic gene *Bcl-2* are almost unaffected by the corticosteroid. In contrast, dioxins cause a more prolonged atrophy of the thymus that is only slightly ameliorated by expression of the *Bcl-2* gene. Indeed, the kinetics of dioxin-induced thymic atrophy are more similar to those caused by estrogen even though the hormone and the toxin act on completely different receptors (Lai et al. 2000; Silverstone et al. 1994; Staples et al. 1998a).

In addition to its effects on thymus tissue, estrogen has many other immunomodulatory actions, including inhibition of spleen T-cell activation, up-regulation of specific cytokines including interferon-γ and interleukin 4, and an antiproliferative effect on blood stem cell precursors. Could mimicking, disruption, or exacerbation of these actions explain sex differences in immune responses to environmental agents, or the disproportionately high incidences of autoimmune diseases in women? Some autoimmune animal models reveal sex-specific patterns of disease and susceptibility to chemical agents. New Zealand black mice have an autoimmune response that leads to red blood cell lysis and kidney damage; males and females are equally affected. But crossing these animals with normal Swiss Webster mice generates female offspring that develop a fully penetrant lethal nephritis—causing death within 32–40 weeks—whereas the male offspring develop very mild kidney damage, do not start dying until 14 months of age, and have no evidence of end-stage renal disease or autoimmunity in the first year of life. However, when Silverstone and Jerrie Gavalchin of Cornell University (Ithaca, NY) administered estradiol or dioxin once a month to these male mice, they did develop renal disease and just as rapidly as did their female littermates (Silverstone AE, personal communication).

Silverstone used radiation chimeras to examine the contribution of specific cells to

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the estrogen or dioxin response. In these chimeras the host mouse is completely irradiated, leaving the animal without hematopoietic stem cells or a functioning immune system. The immune system is then reconstituted by injecting bone marrow cells from a nonirradiated donor. The latter, however, can be engineered without specific receptors, such as those for estrogen or dioxin. When such animals are used as donors, the resulting chimeras lack those receptors in the immune system but express them normally in other tissues (Gasiewicz et al. 2000).

Such chimeras have allowed Silverstone and colleagues to conclude with certainty that the dioxin-induced thymic atrophy is mediated by direct activation of the receptor in a small population of developing immune stem cells (Staples et al. 1998b). Similar experiments show that chimeras lacking estrogen receptor α (ERα) in the immune system are less sensitive to thymic atrophy induced by estrogens, but even more surprising was the discovery that a lack of ERα in nonhematopoietic cells produces attenuated thymic atrophy (Staples et al. 1999).

Women diagnosed with autoimmune diseases suffer the burden of this interplay between hormones such as estrogens and the environment. For example, the incidence of systemic lupus erythematosus, one of the most common autoimmune diseases, is 10–15 times higher in women than in men (Lupus Foundation of America 2003).

The exact causes and triggers of autoimmune reactions are not fully understood, but there are good indications that environmental exposure to infectious and environmental agents can play a crucial role. For example, autoimmune myocarditis, one form of which used to be known as rheumatic heart disease, can directly result from an untreated streptococcus infection (Dell et al. 1991). In another example, contaminated cooking oil was found to be responsible for an outbreak of scleroderma-like symptoms among women in Spain (Navas-Palacios et al. 1984).

Environmental hazards to the immune system include heavy metals (Lawrence and McCabe 2002), and more specifically, there is an increasing body of evidence linking mercury to autoimmunity (Nielsen and Hultman 2002). For example, there are reports showing scleroderma patients who have the most severe symptoms and highest anti-fibrillarin titers, a marker of the disease, also have higher levels of urinary mercury than do their less symptomatic cohorts (Arnett et al. 2000). Such studies have led Ellen Silbergeld of Johns Hopkins University (Baltimore, MD) to focus her attention on the role of mercury in autoimmune diseases.

Silbergeld has found that mercury has a profound impact on graft versus host disease (GVHD) models of autoimmunity that mimic lupus (Via et al. 2003). In these models, GVHD is induced by injecting splenocytes from one strain of mice (C57BL/6 or DBA/2) into hybrid offspring (C57BL/6 × DBA/2). Injecting the latter with splenocytes from DBA/2 or BL/6 mice leads to chronic and acute lupuslike disease, respectively, and these mice usually start to die about a year after the treatment. However, if inorganic mercury is administered at concentrations as low as 20 μg/kg to both the graft donor and host before the grafting, the disease is accelerated; 70% of these animals are moribund after 2 months and all die within 4 months. Hosts compromised by mercury also show significantly elevated proteinuria—up to four times that of controls—and histopathologic examination of their kidneys shows severe lupuslike tissue damage not seen in sham-grafted animals. Significantly, the latter appear unaffected by these low doses of mercury, whereas GVHD animals die of lupus—not of mercury poisoning. Furthermore, Silbergeld and colleagues observed stark sex differences in the response of the hosts, males being unaffected by the metal.

Mercury also has a profound effect in models of cardiac myocarditis (CM). In autoimmune CM, cardiac myosin is the major antigen that is targeted by the immune system. In fact, inoculating animals with this protein can induce the disease. Mercury-treated mice develop cardiomyopathy, have impaired cardiac function, and show elevated levels of cardiac myosin-specific IgG. If these animals are given mercuric chloride (10 or 100 μg/kg) before induction of CM, all these symptoms are exacerbated, whereas control animals appear unaffected by the metal. Precisely how mercury modifies the immune response is unclear. It may act as an adjuvant, suggested Silbergeld, boosting autoimmune responses in those already susceptible, or it may lead to overactivation of lymphocytes. Sibergeld's data indicate that at relatively low doses, inorganic mercury has profound effects on autoimmune responses, suggesting that toxicity studies should be carried out in the context of "co-exposures." Such studies could be conducted in animals that are susceptible to autoimmunity.

These and similar experiments are paving the way for additional studies that are needed to elucidate immunotoxic effects of a variety of chemicals in the environment. Essential to our understanding of the interaction of the hormones and the environment is the identification of specific cellular targets for toxic agents that cause immune effects.

Heavy Metals and Steroid Receptors

Other heavy metals may also have significant biologic effects. The research of Mary Beth Martin, of Georgetown University Medical Center (Washington, DC), has revealed that divalent cations, including cadmium, copper, vanadium, cobalt, mercury, and tin, may all mimic to some extent the effect of estradiol on cells expressing ERs. Martin and colleagues have extensively studied the estrogenic-like effects of cadmium in cell cultures and in mouse model systems. Cadmium chloride, they have found, will promote growth of the MCF-7 human breast cancer cell line, cells that are normally amitotic in the absence of estradiol. This growth is accompanied by a decrease in expression of ERα and increases in levels of the progesterone receptors PS2 and cathepsin D, responses that are typical of growth induction by estradiol (Garcia-Morales et al. 1994).

But how exactly does cadmium elicit these responses? Signal transduction through the ER requires a translocation of the hormone/receptor complex to the nucleus, where it can bind to estrogen response elements that bestow estrogen sensitivity on target genes. Cadmium could conceivably act anywhere along this pathway, or it could act indirectly through nonspecific interactions that impinge on estrogen signal transduction. The evidence, however, overwhelmingly favors the former scenario. The estrogen antagonist ICI 164384, for example, attenuates the effects of cadmium on gene expression. In MCF-7 cells, either estradiol or micromolar levels of $CdCl_2$ will induce a 4-fold increase in expression of the progesterone receptor, but both of these actions are blocked by the antagonist. Furthermore, in COS-1 cells, which are devoid of ERs, chloramphenicol acetyltransferase (CAT) reporter genes engineered with estrogen response elements are activated by cadmium only when the cells are also transfected with ER genes, clearly implicating the receptor in cadmium signaling (Stoica et al. 2000b).

ERs, like the rest of the steroid hormone receptor family, have a hormone-binding domain and a DNA-binding domain that is dominated by zinc-finger motifs. The presence of this other heavy metal in the receptor suggested that this may be where cadmium could introduce itself into the signal transduction pathway. As it turns out, the zinc-finger domain is dispensable for cadmium's actions. Martin demonstrated this by using COS cells transfected with chimeric receptors that contain an estrogen-binding domain linked to a GAL4 DNA binding domain. When these cells are challenged with estradiol, the chimeras induce expression of GAL-driven CAT reporters. A similar induction is achieved with micromolar levels of cadmium, and both responses are blocked by estrogen antagonists (Stoica et al. 2000a).

These findings indicate that cadmium binds to the estrogen-binding domain of the receptor. But although Scatchard plots show that cadmium's affinity for the receptor is

quite high (dissociation constant is 10^{-10} M), they also indicate that the metal and steroid do not compete for binding to the receptor. Likely sites where the metal could bind include some that are near aspartic acid, glutamic acid, and histidine residues, which could provide the necessary ligand field to chelate the atom. Crystallographic data predict several likely binding sites, and by mutational analysis Martin confirmed the role of glutamine 523, histidine 524, and aspartic acid 538 in mediating the estradiol-like action of the metal—introducing an alanine in each of these positions that has no impact on estradiol activity but prevents activation of the receptor by cadmium.

These amino acids lie in a loop between alpha-helices 11 and 12 of the hormone-binding domain. Helix 12 is particularly important because in the absence of the steroid it binds to heat shock proteins. These keep the hormone-binding pocket open and prevent the binding of co-activators, which are necessary for activation of transcription. Martin predicts that when cadmium binds to the loop between helices 11 and 12 the heat shock proteins are displaced, allowing co-activators to bind, turning the ligand-free receptor into a potent transcription messenger.

The action of cadmium is not restricted to cultured cells. *In vivo*, at levels approaching environmental exposures, the metal has profound effects on uterine and breast tissue. In ovariectomized mice given a single intraperitoneal dose of cadmium (2 μg/kg), the uterine size almost doubled after 4 days. These results are comparable with those in animals receiving hormone replacement. Similarly, mammary gland tissue increased by about 50% 4 days after administration of the metal but after 14 days increased more than 30-fold. Cadmium also affects mice *in utero*. As little as 0.5 μg/kg given to pregnant females at day 12 of gestation causes acceleration in the development of the reproductive organs of the pups (Johnson et al. 2003).

Martin has found that cadmium can also activate androgen receptors. LN CaP cells, a prostate cancer cell line that requires androgen for growth, will proliferate in the presence of cadmium. In a series of experiments that parallel those described above, Martin and colleagues have shown that Cd^{2+} also binds to the androgen receptor with high affinity and noncompetitively with respect to free androgen (Martin et al. 2002). These findings could explain recent epidemiologic observations that correlate elevated levels of the metal in benign prostatic hyperplasia samples (0.98 μg/g Cd^{2+}) and prostate tumors (8.8 μg/g Cd^{2+}) versus normal tissue (0.7 μg/g Cd^{2+}).

The effects of cadmium on mammary glands and the prostate raise the question of what other organs and tissue may be adversely affected by this metal. Gonadal steroid receptors are found in many nonreproductive organs including the liver, blood vessels, and brain. Further research is needed to elucidate the impact of mercury, cadmium, and other toxins acting through this mechanism.

Cancer and the Environment

Although cadmium may be a factor in breast and prostate cancer, the exact cause of most human cancers is unknown. Environmental exposure to varied and multiple carcinogens does play a role in the growth of some cancers, but the fact that some smokers die early of lung cancer whereas others live to old age to die of some other illness indicates that other factors must predispose certain individuals to cancer. These predispositions or individual susceptibilities could result from sex, race, socioeconomic status, or behavioral or genetic differences. Age may play a role, but the suggestion that accumulating carcinogens or carcinogenic insults are responsible for the age-related increase in the incidence of cancers is challenged by the fact that after middle age the increase in cancer incidence slows. Nevertheless, these three factors—predisposition, age, and environment—have been used to explain carcinogenesis in the simplest terms (American Cancer Society 2003). Although predisposition and age cannot be altered, primary prevention may be achieved only by reducing environmental exposure. This fact alone should make the identification of chemical carcinogens a priority (Schmahl 1988).

James Huff, from the NIEHS, and colleagues use animal models of experimental carcinogenesis to identify potential human carcinogens. These studies supplement, and most often precede, epidemiologic, clinical, and other studies, such as those on cancer clusters or occupational or environmental exposures, which are reported for human populations.

Chemically induced carcinogenesis in animals must be carefully evaluated, however, because the response to a given chemical may occur only in a tissue or organ that has no counterpart in humans, or in a particular species or sex, the latter being vitally important because it often indicates sex hormone mediation. Years ago there was almost universal consensus that both sexes of an animal had to elicit the same carcinogenic response from a chemical or the result was considered suspect. In addition, although some of the most common human cancers, breast and lung, for example, can be induced in animals, there are also some human tumors, including prostate and colorectal, that are not typically observed in rodents.

Huff stressed that all human carcinogens that have been tested in animal bioassays are also carcinogenic in animals. Further, nearly one-third of the identified human chemical carcinogens were first discovered in animals and only later in humans, adding considerable support for the validity of animal bioassays. Despite these findings, the use of bioassay results to predict the public health significance of environmental exposures remains controversial.

As Huff outlined, predicting carcinogenesis on the basis of chemical activity, such as mutagenicity, toxicity, or structural formula, is not a fully reliable methodology. He and colleagues used this approach for 400 different chemicals. Of 267 chemicals suspected strongly of being carcinogens, only 181 (68%) returned positive evidence in bioassays. This result points to the inherent difficulties in predicting chemical carcinogenesis and strengthens the rationale that animal studies are essential for protecting human health (Fung et al. 1995).

The issue of sex differences is difficult to address in these model systems because there are not always animal correlates to human cancers. Animals almost never get prostate cancer, for example, but appropriate models are being developed. Methylnitrosourea is a known prostate carcinogen, and it would be interesting, Huff postulated, to expose strains of rats and mice to this agent to find an animal that would be a good model for prostate-specific carcinogens. However, such models may be fraught with pitfalls. For example, if one were to test a mammary gland carcinogen on a prostate model and nothing happened, how would one interpret the data? Single-organ models may be most useful for discerning mechanistic insights or for confirming human evidence. The two-species, two-sexes bioassay should remain the accepted approach to identifying environmental and human carcinogens.

Kent Hunter, of the National Cancer Institute (Bethesda, MA), has done similar work on this theme by exploring genetic and environmental factors that influence sex-specific cancers. Hunter and colleagues use transgenic mice that develop multifocal mammary tumors to study the genetics and environmental influences on breast cancers and their metastases. These mice express the polyoma middle T gene, which causes cancer by 9 weeks and rampant metastatic disease by the time the animals are 100 days old.

In genetic screens Hunter and colleagues crossed their highly tumorigenic mice with normal inbred mice (> 25 strains) and examined their offspring for cancer and metastases. Remarkably, some of these offspring had tumors that were very poor at metastasizing, and by back-crossing these animals with their cancer-free parents, Hunter and colleagues tried to pinpoint the genes responsible for this trait. Crosses and back-crosses carried out with different strains revealed common "hot spots" on several chromosomes, including

9, 13, 17 and 19, that link to the nonmetastatic phenotype. In chromosome 9, one of these "hot spots" contained two genes that are known to be involved in cancer: the tumor suppressor gene *ATM* and the gene for Chk1, which functions in the repair of damaged DNA (Hunter et al. 2001; Lifsted et al. 1998).

Hunter and colleagues isolated and sequenced these genes and found many polymorphisms across strains that may explain the varying numbers of metastases seen in the original crosses. One way to test this hypothesis would be to make transgenic knockouts that lack either or both of these genes. An alternative test would be to use a drug or chemical that inhibits ATM or Chk1. Such molecules have already been reported in the literature and include caffeine.

Hunter and colleagues examined the effect of caffeine on the transgenic mouse model by administering drinking water with a caffeine concentration typically found in green tea. Mice were given the stimulant either at weaning or after the first tumors developed (~60 days). Both regimens reduced the total tumor burden, although this was statistically significant only for animals taking the stimulant upon weaning. Furthermore, the metastatic potential of the tumors was significantly reduced in both groups of animals (Yang H, unpublished data).

This effect is actually opposite to what one would have predicted if a tumor suppressor were being inhibited. One explanation for these surprising results could be that caffeine suppresses the ability of the primary tumors to form, thus lowering the total number of tumors and, concomitantly, the number of metastases. Hunter tested this hypothesis by examining the histology of the mammary glands. But instead of seeing a reduction in the number of tumors, what was observed was a complete transformation of the mammary tissue.

Carcinogenesis is a complex, multifactorial process rooted in the interaction of an organism with its environment. Sex is just one of the factors that can determine whether a potentially carcinogenic exposure results in disease. Elucidating the roles and interaction of carcinogens with gonadal steroids and their receptors and target tissues is critical to a fuller understanding of carcinogenesis.

Endocrine Disruptors

There are almost 100 known endocrine-disrupting chemicals (EDCs) that may alter the function(s) of the endocrine system and consequently cause adverse health effects in intact organisms, their progeny, or subpopulations. Such disruptors include many commonly used chemicals, such as organohalides, pesticides, and phthalates that are used in plastics. In addition to the known endocrine disruptors, the U.S. Environmental Protection Agency (EPA) estimates that there are > 80,000 potential EDCs that need to be evaluated for endocrine effects (U.S. EPA 2003).

Some of these effects are sexually dimorphic. In females EDCs have been linked to impaired fertility, increased incidences of uterine and fallopian tube abnormalities, breast and reproductive tract cancers, and endometriosis (Hodges et al. 2000; Iguchi et al. 2001; Safe 2000). The latter can be induced in primates by extremely low levels of dioxin, about 25 ppt (Rier et al. 1993, 1995). In males EDCs have been linked to declining sperm counts (although this has been hotly debated), increases in incidence of prostate and testicular cancer, and deformities of the reproductive tract; the incidence of the latter has doubled over the last 20 years. In both sexes EDCs can affect the immune system, lead to behavioral changes, and have multigenerational effects (Damstra et al. 2002).

Although, by definition, all EDCs act on the endocrine system, they may act in very different ways. They can alter enzyme action, bind to receptors, affect metabolism and hormone availability, or alter gene expression. All this is complicated by the fact that natural systems are also heterogeneous and have, for example, multiple closely related hormones (e.g., testosterone and dihydrotestosterone) and receptor subtypes (e.g., ERα, ERβ). There are also species-specific hormone variations and effects that need to be considered. Whereas testosterone is the predominant androgen in most vertebrates, 11-ketotestosterone is used by fish. Prolactin in humans and other mammals promotes milk production by mammary tissue, but in amphibians it helps regulate metamorphosis and is necessary for limb regeneration.

Dose relationships are not easy to interpret, either, because some EDCs do not have typical monotonic dose–response curves. In rodents, increasing doses of diethylstilbestrol to 0.2 μg/kg, for example, produces larger prostate sizes, but if the dose is increased much further, the prostate mass drops below that of controls (vom Saal et al. 1997). Furthermore, when animals are exposed to more than one EDC, the impact may be much greater than the sum of individual exposures (Silva et al. 2002). However, because the endocrine system regulates anatomical development, a single low-dose exposure can have major consequences.

Critical windows for different organ systems also vary considerably. In humans, for example, although the major limbs are fully developed by 10 weeks, the central nervous system and genitals continue developing right up to birth. In amphibians, the discovery of multiple limbs and digits caused great public and scientific concern. Wildlife biologists were initially stumped by these deformities because no endocrine disruptor could be found to elicit such altered patterns of development. Subsequently, it was found that the deformities can be caused by trematode infections, and that exposing the amphibians to endocrine disruptors, such as those found in agricultural runoff, increases their susceptibility to the parasite and to developmental abnormalities. Indeed, in frog populations exposed to trematodes, concomitant agricultural runoff causes four to six times more deformities compared with populations not exposed to runoff (Kiesecker 2002).

Brent Palmer, of the University of Kentucky (Lexington, KY) has found that developmental susceptibility also arises in salamanders, which are sensitive to a wide range of EDCs, including atrazine, carbaryl, endosulfan, and octylphenol. His group has found that embryonic exposure to these chemicals may cause increased mortality, reduced growth, and behavioral problems (Rehage et al. 2002).

Salamanders must balance their time between foraging for food and seeking shelter from predators. Endosulfan (10 ppb), octylphenol (500 ppb), and atrazine (400 ppb) significantly alter activity patterns in salamanders. Endosulfan and octylphenol make the animals lethargic, preventing them from foraging for food. In addition, the EDC-treated animals show a greater tendency to move after being disturbed. Exposure to 400 ppb atrazine, for example, nearly doubles the chances that a salamander will respond to a disturbance by moving instead of seeking cover. In natural settings, this leaves the animals open to attack by predators (Rohr et al. 2003) and has the possibility of wiping out the population.

These experiments reveal the profound impact that endocrine disruptors can have in the wild. Palmer and colleagues are presently building computer models to study how these and similar effects can affect whole populations. The impact can also be multigenerational, he reminded workshop participants.

For example, studies have shown that for humans exposed to PCB-contaminated cooking oil, the sex ratio of their offspring was dramatically altered. The proportion of boys dropped from an average of about 55% in the normal population to as low as 40% in those exposed to PCB (del Rio Gomez et al. 2002). Other population effects may go unreported, suggested Palmer, because the Gaussian distribution of a particular response is not shifted left or right by exposure to a disruptor, but instead the curve tends to flatten because of increased variation. Those affected are often the outliers in the curve, he theorized, and unless the sample numbers are very large this fact may go undetected.

Bernard Weiss, University of Rochester School of Medicine and Dentistry (Rochester, NY) also reported that there is considerable sexual dimorphism in behavioral responses to

endocrine disruptors. Low levels of dioxin had not previously been shown to affect wheel-running patterns in rodents, but Weiss and colleagues delved further into these behaviors and made some interesting observations.

In normal rats, wheel running is a sexually dimorphic behavior. In females, the amount of running is correlated with the stage of the estrous cycle, and they also run more than their male littermates. Weiss and colleagues set up an experiment in which the animals were trained to press a lever a specific number of times to gain access to a single period of activity on the running wheel. The number of times the lever had to be pressed varied from one (fixed ratio of one; FR1) to 30 (FR30). The researchers adopted this situation to measure the motivation to run rather than simply measuring spontaneous running. To test his hypothesis that dioxin influences female brain development, Weiss gave pregnant mothers a single dose of the dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; between 0 and 180 ng/kg) at day 18 of gestation. When the offspring were 5 months of age, they were trained and tested in the modified running wheel.

Weiss and colleagues found that the dioxin-treated female rats pressed the lever fewer times and earned fewer opportunities to run than did controls. Significantly, the dose–response curve for this outcome suggests that an ED_{10} (the dose required to produce a 10% change in performance) would be about 7 ng/kg, within the range of the known dioxin burden of the human population that was calculated to average 13 ng/kg in 1995 based on the sum of TCDD and related agents (Markowski et al. 2001).

Weiss and colleagues also used a schedule-controlled operant behavior regimen to test the effects of the same exposure to dioxin but at day 8 of gestation. In this experiment, the animals received a reward for tapping a lever a set number of times (which was progressively raised from 1 to 71) or, on another schedule, for waiting at least 10 sec between successive lever responses. Normally, males press the lever more frequently than their female littermates. In this experiment, however, dioxin had the opposite effect on male and female offspring. Males from treated mothers responded at lower rates than did males of untreated mothers, whereas females of treated mothers responded at higher rates than did control females. At intermediate doses of dioxin (60 ng/kg), the dioxin-treated female offspring actually outperformed control males (Hojo et al. 2002).

These sex differences in response to dioxin may be related to differences that already exist in the animals. In humans, male brains are known to be more asymmetrical and to have proportionately more white matter than those of their female counterparts, whereas in rats males have thicker right cerebral cortices, higher cell counts in the right hemisphere, and more ERs in the right neonatal cortex. These biases are all reversed in female animals whose mothers were given dioxin. Could the effect of dioxin on female rats be caused by altered patterns of development in the brain? Weiss and colleagues examined the depth of the cerebral cortices in offspring of rats that had been treated at gestation day 8 with the highest levels of dioxin (180 ng/kg) and found that the normal lateralization—thicker left hemisphere—was reversed, indicating that the female brains had been "masculinized."

Genomics

In exploring sex differences such as those discussed in this article, the ultimate goal must be to elucidate the biologic pathways or activities that are affected by environmental exposure. Before the genomics era, this task seemed insurmountable, but the recent completion of the human, mouse, yeast, and other model genomes, coupled with the arrival of a suite of genomic and proteomic tools that accelerate the accumulation of biologic data, has provided the means and opportunity to carry out the required experiments.

Mary Jane Cunningham, currently with the Houston Advanced Research Center (Houston, TX) and formerly from the Molecular Mining Corporation (Kingston, Ontario, Canada), has used genomic tools to probe the effects chemicals have on gene expression in both male and female rats. By comparing cDNA libraries made from treated and untreated animals, Cunningham and colleagues have shown that administration of prototypical hepatotoxins, such as carbon tetrachloride, fenofibrate, and hydrazine, along with less toxic chemicals such as acetaminophen and clofibrate increases expression of specific genes in the liver. Some of these genes, such as those for cytochrome P450 and acetaminophen-binding protein, have a known function, but many do not. By carrying out subtractive hybridizations on samples from male and female livers, Cunningham was able to identify sex differences in the response to these chemicals. For example, both benzo[*a*]pyrene and clofibrate cause the same gene of unknown function (g2224669) to appear more abundantly in females than in males.

DNA microarrays have allowed Cunningham and colleagues to compare and contrast hepatocyte gene expression patterns in animals that have been treated *in vivo* with chemical agents. Acetaminophen, benzo[*a*]pyrene, and clofibrate, three compounds that affect different metabolic and detoxification pathways, cause significant up- or down-regulation of 269, 146, and 271 genes, respectively, out of a total of 7,400 selected for the array. Although the expression profiles for each of these treatments were unique, some commonly regulated genes exist and may represent toxicity markers, but these were few. Of the 172, 186, or 60 genes that were affected by acetaminophen, benzo[*a*]pyrene, or clofibrate, respectively (after more stringent filtering of the data using the signal of the arrayed spot ≥ 40%, ratio of signal to background ≥ 2.0, and spot intensity ≥ 250), only nine (or 3%) were found to be common.

Microarray analysis is also a useful tool for analyzing how responses to drugs or toxic agents differ between males and females. Cunningham has found that when rats are challenged with clofibrate, several genes are up-regulated and down-regulated in female livers compared with their male peers, a result that was mirrored by analysis of the liver proteome. It is noteworthy, perhaps, that of the genes that are disproportionately down-regulated in female liver tissue, one is the liver-regeneration–related protein, which is involved in the early liver regeneration response.

Although such high throughput techniques hold the potential for advances in the field of genomics and environmental health, there is concern that much of this technology and the data garnered from using it remain in databases belonging to proprietary firms. Industry should partner with agencies such as the NTP (Research Triangle Park, NC) to create transparent databanks that can be shared among researchers at the earliest stage possible. Such cross-industry collaboration will speed scientific discovery and potentially affect the health of millions of people as potential toxins are identified.

Conclusions

By the end of this decade, experts predict that more than 100,000 chemicals will be registered for commercial use in the United States (NTP 2002). We come into contact with these chemicals through the food we eat, the air we breathe, the medications we take, and the clothes we wear. We are continually learning more about how these compounds interact with the body and the long-term impact of these interactions on health. The emerging field of sex-based biology is revealing that these interactions can differ between women and men.

Environmental exposures can affect organisms from cellular function through behavior. New research approaches are needed for us to understand better the interaction of these exposures with biologic sex differences. Crucial to the success of such scientific exploration is hypothesis-driven research and the support of investigator-initiated research by federal agencies. Additionally, the Society for Women's Health Research calls on environmental health

researchers to include sex as a variable in all data collection, including data derived from cell lines and tissue cultures.

To fully understand the potential effect of chronic exposures on the sexes, researchers need to develop models to explore not only physiologic sex differences but also behavioral responses to low-dose and multiple chemical exposures. Additionally, researchers should track these responses across multiple generations to identify potential long-term risks of exposures and the influence of sex on that risk. Incorporated in this approach should be a better understanding of which end points one needs to examine. A multidisciplinary approach to environmental health research is central to fostering discovery. As we learn more about the mechanisms of environmentally related diseases, we see that exposures can affect the individual across multiple organ systems. Only by working across disciplines will we be able to fully understand the impact of the environment on health.

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