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The emerging science of endocrine disruption

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In this essay we provide an overview of the emerging science of endocrine disruption. We begin with a brief definition and a consideration of several inter-related concepts which are forcing important conceptual shifts into the theory and practice of toxicological science. We then examine some of the empirical bases for these conceptual shifts, first from studies of wildlife, then from studies with laboratory animals. In a final section, we shift to a consideration of the considerable challenges this new view of toxicology creates for human epidemiology. Some progress, however, is being made towards a more “environmentally-sensitive epidemiology” which we describe briefly and illustrate with a recent example relating to marked regional differences in sperm count in men living in the USA.

Background

Definitions

All living organisms depend upon a large and intricate array of chemical signaling systems to guide biological development and regulate cell and organ activity (McLachlan 2001). Over the past two decades, scientific interest in the ability of many environmental contaminants to interfere with these sensitive systems has grown dramatically. A hybrid science, the study of endocrine disruption, has arisen from concerns about the effects of these phenomena on health and the environment (Colborn et al. 1996). This science incorporates findings and methodologies from multiple disciplines including toxicology, endocrinology, developmental biology, molecular biology, ecology, behavioral biology and epidemiology.

Endocrine disrupting chemicals (EDCs) are chemicals that can disrupt thyroid hormones, androgens, estrogens and other endocrine processes. EDCs disrupt development by interfering with the hormonal signals that control normal development of the brain and other organ systems. EDCs can also affect adults by similar mechanisms because these same hormones also play important regulatory roles in adults (Colborn et al. 1993; Colborn et al. 1998). EDCs can act at very low levels of exposure to produce profound effects on the course an organism follows from fertilized oocyte through to maturity, adulthood and death. The effects of EDCs on developing organisms are of greatest concern, since the disruptive effects of developmental exposure are permanent and irreversible—termed organizational effects—whereas EDC exposure produces measurable, activational effects in adults that may be

reversible. A related field of research, “developmental origins of health and adult disease” is converging with research on endocrine disruption to consider how exposures during different stages of development, particularly during fetal life, contribute to adult chronic diseases including obesity, heart disease, diabetes, decreased fertility, impaired immune function and neurological deficits.

Data accumulated over the past two decades reveal substantial global contamination by EDCs. Contaminant dispersal is brought about by a combination of factors, including purposeful or accidental release into the environment followed by long-range atmospheric transport. It also occurs because some EDCs have been incorporated inadvertently into consumer products. With regard to long-range transport, large masses of air have been tracked across the Pacific carrying a variety of pollutants from central Asia to the west coast of the US virtually undiluted, including ozone, heavy metals and organochlorine compounds. In addition, so-

called “global distillation” processes—repeated sequences of volatilization and condensation—transport semi-volatile compounds from sites of production, use and disposal to colder regions, particularly at high latitude and altitude.

Two of many examples of inadvertent contamination of people due to the use of consumer products include exposure to phthalates and bisphenol A (Myers 2003). Phthalates are used as additives in cosmetics, intravenous tubing and other polyvinyl chloride (PVC) plastics. Polyvinyl chloride products contain phthalates to soften the otherwise brittle PVC. Exposure to bisphenol A is also widespread. Bisphenol A is a monomer (not just an additive) used in the manufacture of resins that line the inner surface of food cans (over 100 billion manufactured per year in the USA alone), and to manufacture polycarbonate plastic, which are used to make food and beverage containers. Phthalates and bisphenol leach from these products and disrupt endocrine function.

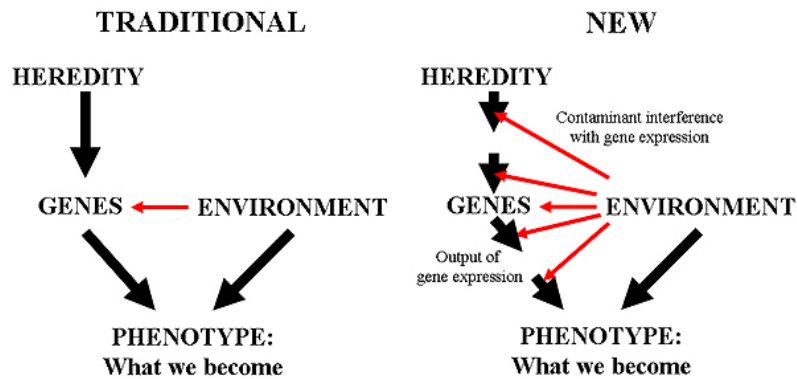


Fig 1. Contrasting traditional with new formulations of the interactions of genes and environment in the determination of phenotype. Traditionally, genetic diseases have been seen as determined by heredity. In the new formulation, genetic patterns of gene expression are vulnerable to disruption by environmental contaminants at multiple points in the sequence of steps that lead to gene expression, thereby rendering genetic diseases susceptible to modification by environmental factors.

Coincident with emerging knowledge of the ability of EDCs to disrupt a range of developmental processes, a series of emerging human epidemics have been reported. These include increases in the frequency of preterm birth, obesity, cognitive/behavioral dysfunctions (such as autism and attention deficit hyperactivity disorder, ADHD), and decreases in reproductive function (such as a decline in sperm count) and immune function. The strength of the epidemiological evidence demonstrating these epidemics varies. There is little argument that there has been a wide-spread increase in rates of obesity and diabetes, but there is still significant debate about global decreases in reproductive function or increases in ADHD, due to limitations of historical data. While extensive study will be required to identify causes of these trends, their underlying biology suggests that alterations in inter- and intra-cellular signaling processes may be causally involved, and for each of the mentioned epidemics, data are available indicating one or more points of vulnerability to EDCs in the mechanisms of control. EDCs may also contribute importantly to geographic variability in these health endpoints. Demonstration of such variation in semen quality and its relationship to current-use pesticides as a probable cause is one such example, discussed further below.

Theoretical concepts

New scientific findings on these issues are emerging at an exponential rate. Central to these findings is a reformulation of the traditional dichotomy between nature and nurture (the gene vs. environment argument) in the causation of disease (Figure 1).

In the old formulation, that which is “nature” is based on genes, while “nurture” comes from the environment, *sensu latu*. Functional status and disease linked to genes have been perceived as completely determined by heredity. Diseases traditionally viewed as non-hereditary (“environmental”) can be caused by a wide array of exposures, stressors, experiences, nutrition and other life-style factors. Concern about environment’s interaction with the genetic determination of disease and functional differences has focused traditionally upon two pathways: 1. high-dose chemical exposures causing mutations and thus alterations in the base sequence of genes, and 2. genetic variation among individuals leading some to be

more susceptible than others to certain contaminants.

The study of endocrine disruption today is turning this historical conceptualization on its head. Rather than simply being a factor determined by inheritance, a property linked to a gene is one that is vulnerable to environmental disruption, particularly by EDCs. This is because EDCs acting at low levels can act by interfering with gene expression and other cellular activities. Clearly some functional deficits and disease states are due to inherited mutations in genetic makeup, but many more diseases may be associated with alterations in gene expression.

Initially, the majority of research on EDCs focused on interference with gene activation by the hormone 17 β -estradiol (the most potent endogenous estrogen). Many EDCs can stimulate genes and other cellular processes in a manner similar to estradiol, whereas other EDCs antagonize estradiol or block the synthesis of estradiol (Welshons et al. 2003). However, over the last decade, EDCs have been shown to disrupt many other endogenous hormonal signaling molecules, including virtually all steroid hormones that have been carefully tested, as well as thyroid, retinoid, leptin, some transcription factors, growth factors and other molecules not traditionally classified as hormones. One recent study even documents interference with chemical signaling between two symbiotic organisms, the bacterium *Rhizobium* and its leguminaceous host (Fox et al. 2001). The presumption now is that any chemically-mediated signaling system is vulnerable, in principle, to disruption by chemicals to which wildlife and humans are exposed to in their daily lives.

Given the enormous potential for EDCs to interfere with gene expression, how many of the 80,000+ chemicals registered for commercial use have endocrine disrupting activity? The vast majority of chemicals have not been tested in even the most basic way. Far fewer have been tested for endocrine disrupting effects, particularly during embryonic development, the most vulnerable time in life

Altered gene expression during organismal development can induce dramatic changes in developmental outcomes, that is, the disruption is irreversible. Known effects of EDCs range from structural changes to

functional deficits. For example, alterations in the production of hormone receptors in tissues through the alteration in the expression of genes for these receptors has been shown in experiments with laboratory animals, and these changes can then lead to altered responses to hormonal stimulation throughout the remainder of life. This can, in turn, lead to altered (increased or decreased) susceptibility to contamination with hormonal activity (Gupta 2000; Richter et al. 2000).

Altered gene expression and cellular signaling subsequent to development can cause transient changes, termed activational responses, or particularly through carcinogenesis, permanent detrimental effects. For example, lifetime exposure to estrogen is the best predictor of breast cancer in women, and exposure to EDCs that are “environmental estrogens” could plausibly increase breast cancer risk. Thus, the impact of EDCs will vary depending upon a variety of factors, including when in the life-cycle of the organism exposure occurs, as well as the duration and amount of exposure. Until recently, the great importance of life stage, the very great vulnerability of the embryo, and the fact that consequences of fetal exposure could be entirely different from those seen from adult exposure had not been appreciated.

Collectively, these new data from studies of EDCs are forcing a series of conceptual shifts that undermine long-held assumptions underlying toxicological studies and the applications of results from these studies to developing public health standards.

Foremost among these is a challenge to the operating assumption concerning appropriate dose. Focusing on traditional toxicological endpoints, such as gene mutations, weight loss and death, toxicologists customarily worked at what now are viewed as very high doses, typically in the range of parts per million and parts per thousand levels. New data suggest that extremely low doses of EDCs (in the part per billion and even part per trillion range) can cause measurable and highly significant endocrine disruption. A growing array of studies is revealing changes in gene expression, including both gene suppression and gene activation, as a result of low-level exposure to EDCs. For example, recent work on arsenic, long-established to be toxic at high doses, has revealed that at part per billion levels, arsenic

can interfere with glucocorticoid activation of genes involved in the control of metabolism, response to stress, immune function and the suppression of tumor formation (Kaltreider et al. 2001).

A second important conceptual shift arises from consistent findings that during the life-cycle of an organism, developmental stages are typically far more vulnerable to signal disruption than adult stages. This is thought to occur for several reasons, including the absence of fully developed protective enzyme systems and higher metabolic rates. Most importantly, however, the events underway in development involve a series of organizational choices that are irreversible once the “choice” in development is determined. In sharp contrast, in adults, the processes at play can very often be reversed by removing the EDC thus returning gene expression levels and organ functioning to normal; these transient effects are termed “activational” effects. One recent example documenting extraordinary differential sensitivity of adult versus. developing life-stages was presented by Hayes et al. (2002), finding adverse effects in tadpoles at 1/30,000th the lowest concentration of atrazine, a herbicide, found to produce adverse effects in adults.

One clear implication of this focus on low level exposure during fetal and neonatal development is that levels of exposure that have been dismissed as “background” and thus “safe” can have deleterious effects. This had never been realized due to the absence of any studies using these low doses combined with virtually no studies of developmental effects at any dose level. Many laboratory studies now support the conclusion of high sensitivity of the embryo and neonate, as do some epidemiological data from human studies. For example, a series of studies of children born to Dutch mothers, exposed to polychlorinated biphenyls (PCBs) and dioxin through consumption of fish and other food in Dutch markets, have shown that low parts-per-billion concentrations of these contaminants impair cognitive (Koopman-Esseboom 1996) and immune system development (Weisglas-Kuperus 2000). One reported consequence of exposure is a shift in the pattern of play behavior in boys toward patterns more typical of girls (Vreugdenhil 2002).

Toxicological experiments, particularly those used to develop regulatory standards of acceptable levels of exposure to environmental

chemicals have been based upon the assumption that “the dose make the poison”, which implies that high doses invariably cause more harm than lower doses. It has been a surprise to regulators to learn that for hormonally active chemicals, this assumption may not be valid. Endocrinologists and physicians have known for

decades that very high doses of hormones and drugs can block rather than stimulate some responses, resulting in what is referred to as a non-monotonic dose-response relationship (effects initially increase and then decrease with increasing dose).

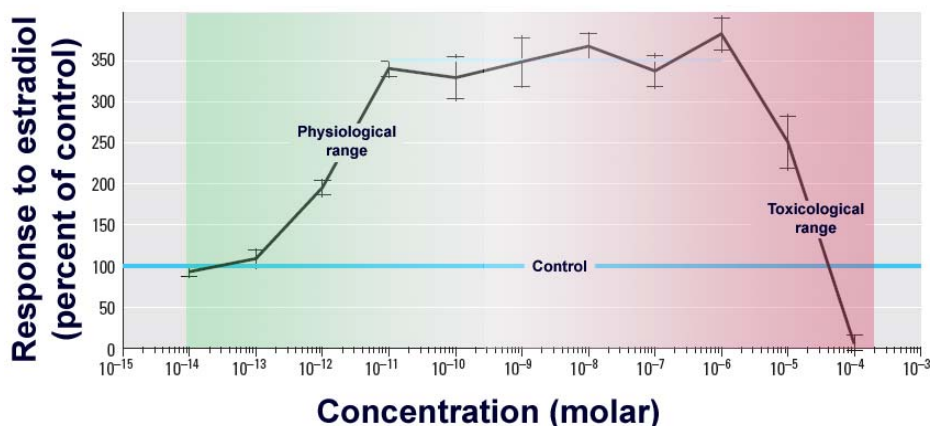


Figure 2. Proliferative response of MCF-7 cells to 17-β estradiol over 10 orders of magnitude. Responses in the “physiological range” are mediated by binding with the estrogen receptor. Those in the “toxicological range” reflect cell death. Adapted from Welshons et al. 2003.

For example, recent work by Welshons et al. (2003), examined effects in response to estradiol exposure in a line of human breast cancer cells (Figure 1). Estradiol levels between 0.1 and 100 parts per trillion produced an increased growth response in breast cancer cells, because at these levels, an increase in exposure causes an increase in the number of estrogen receptors bound by estradiol, thus leading to increased gene activation. At exposure levels in the typical toxicological dose range (part per million range), further increases in the dose of estradiol began to produce cell death. This result is extremely important for regulatory toxicology, because the high level exposures in these experiments are analogous to those used for the prediction of risk posed by low doses, but the actual effects of low doses predicted to be safe have, until very recently, never been examined experimentally (vom Saal and Sheehan 1998). Changes in dose within this very high part per million dose range cannot reveal variations in receptor-mediated gene activation, since all receptors are occupied at doses which are millions of times lower. Hence, testing EDCs at only very high doses is likely to miss signal disruption events that can be

expected to occur at much lower levels of exposure.

Identification of low-dose effects that are different from those seen at high doses, the importance of timing of exposure, recognition of the unique effects that can be disrupted during development, and genetic variation in genetically-determined susceptibility, render the overly simplistic assumptions previously used in risk assessment invalid for many environmental chemicals.

Wildlife and laboratory studies

For many decades, we have been concerned with the effects of environmental contaminants on the health and persistence of wildlife populations. Prior to work over the last 10 to 15 years, the vast majority of these studies examined the lethal consequences of exposure, or they focused on the induction of cancer or major birth defects. Although these endpoints are still critical in the study of toxicology, a growing collection of studies examining diverse wildlife species demonstrates that additional adverse outcomes can be produced in wildlife as a result of exposure to environmental contaminants. A

number of these abnormalities have been attributed to the disruption of endocrine signaling (see Colborn and Clement, 1992; Guillette and Crain, 2000). Below, we examine just a few examples of endocrine disruption in wildlife.

Fish, Vitellogenesis and Sewage

In the 1990s, reports were published documenting that male fish living below sewage outfalls in Europe, Great Britain, North America and Japan had elevated plasma concentrations of the yolk protein vitellogenin (see Sumpter and Jobling, 1995). Naturally, vitellogenin is synthesized in the liver of the female following stimulation by elevated plasma estrogens of ovarian origin. Males of many vertebrate classes, including, fish, amphibians and reptiles, have the ability to synthesize vitellogenin if stimulated by estrogen, although this does not occur normally. Intensive chemical fractionation of sewage identified two major classes of compounds capable of acting as estrogens in male fish, these included the pharmaceutical estrogen, ethinyl estradiol, and the industrial chemicals, nonylphenol and octylphenol. Ethinyl estradiol is a common ingredient of the human birth control pill and is excreted in the urine of females taking this pharmaceutical agent. Ethinyl estradiol has been identified in the surface and reclaimed sewage waters from all continents where such studies have been performed (Kolpin et al. 2002). Similarly, nonylphenol, an alkylphenolic chemical, is widely used in industrial applications as a surfactant and is commonly released into the environment. It is persistent in the ecosystem with very large concentrations found associated with sediments and organic matter in freshwater and estuarine regions. It has been shown to be weakly estrogenic in mammalian laboratory animals, but is a potent estrogen in many fish (White et al., 1994). Laboratory-based life-cycle testing with ecologically relevant concentrations has shown that both of these compounds have adverse effects on the reproductive potential of males and females, and they also alter sex determination in developing embryos (Tyler and Routledge 1998). These common pollutants have the potential to disrupt the health of individual animals and the persistence of populations; some populations have no males. It has also been suggested that endocrine disruption could be associated with the decline of commercial and sport fish populations.

Alligators and Pesticides

Alligators and crocodiles are long-lived top predator species inhabiting most subtropical and tropical wetlands. Studies begun in the late 1980s reported abnormalities in central and south Florida (USA) populations of the American alligator exposed to various contaminant mixtures associated with modern agriculture, such as insecticides, herbicides and fertilizers (Guillette et al. 2000). These abnormalities include altered plasma sex steroid profiles, gonadal, genital and immune tissue anatomy, and hepatic steroid metabolism (Guillette and Gunderson 2001). Specifically, male alligators exposed in ovo (as embryos) to various pesticides, due to deposition in the eggs prior to being laid by the female, exhibit significantly reduced plasma testosterone concentrations, aberrant testicular morphology, and small penis size. Females from the same contaminated locations displayed significantly elevated plasma concentrations of estradiol as neonates but reduced concentrations as sub-adults. Sub-adult females also had elevated plasma concentrations of the potent androgen dehydrotestosterone. They also exhibit a high frequency of polyovular follicles, an ovarian abnormality associated with low fertility and high embryonic mortality. These contaminated populations have shown elevated embryonic mortality greater than 50%. Polyovular follicles are a documented outcome of exposure of women to the estrogenic drug diethylstilbestrol during fetal life exposed as fetuses due to their mothers taking this drug during pregnancy, and these exposed women also suffer a decrease in fertility.

Populations displaying these abnormalities have elevated egg, tissue or serum concentrations of a wide range of organochlorine pesticides or their metabolites, heavy metals and other widely used agricultural chemicals, such as nitrates. Experimental exposure of developing alligator embryos to various organochlorine pesticides or their metabolites induces many of the abnormalities seen in wild populations, such as altered plasma hormone profiles and small penis size as well as altered sex determination (Matter et al. 1998). Concentrations required to induce these abnormalities were in the part per trillion to part per billion range, 100-1000 times lower in concentration than the reported levels in alligator eggs or serum.

Recent studies of Mosquito fish from the same contaminated lakes indicate that the reported abnormalities are not limited to a single

lake or species, as male Mosquito fish have reduced tissue concentrations of testosterone, lower sperm counts and altered reproductive behavior (Toft et al. 2003).

Fish and Pulp Mill Effluent

Many studies have documented the detrimental effects of pulp mill effluent on the environment over many decades. Classical ecotoxicology studies reported wide scale disruption of populations, including the local extinction of many exposed freshwater or estuarine fish and invertebrate populations. Although modifications in the processing of pulp mill effluent have occurred over time, abnormalities persist. Studies from several Canadian locations report altered hormone profiles in fish exposed to pulp mill effluent, including alterations in hypothalamic, gonadal and adrenal hormones (McMaster et al. 1996). Exposed fish displayed altered stress responses and altered reproductive performance.

Masculinization of females has also been reported. For example, female Mosquito fish living below effluent outfalls from paper pulp mills develop a gonopodium, a modified anal fin found in males of this species and used to transfer sperm to the female for internal fertilization (Davis and Bortone, 1992). The gonopodium develops in the male following exposure to androgens, specifically testosterone. Masculinized females do reproduce but have lower production of offspring and greatly elevated levels of aromatase activity in their brain and ovary (Orlando et al. 2002). Aromatase is an enzyme that converts the hormone testosterone to estradiol, the principle estrogen in these females. These females thus have an impaired potential to produce this critical hormone that regulates reproduction.

Fish, Feedlots and Pharmaceuticals

Modern animal production techniques, in many countries, involve the use of potent hormones and antibiotics. Although there has been an on-going debate on the safety of the meat products produced from such practices, few concerns have been voiced concerning the possible ecological impacts of these practices. We have recently examined feral fish exposed to effluent released from animal feedlots, including urine and feces, into a natural river system. We observed that male fish exhibited many of the classical signs of androgen exposure, including reduced testicular mass, reduced plasma testosterone and altered head morphology

(Orlando et al., submitted). With the extensive use of anabolic steroids in cattle production, the potential for wide scale disruption of fish reproduction is possible, since the presence of ethinyl estradiol in rivers after excretion by women and bacterial action in water treatment plants leads to endocrine disruption in fish. Experimental laboratory-based studies support our field observations, as low level exposure to the commonly used anabolic steroid trenbolone alters fish development and reproduction in a manner similar to that observed in the wild fish (Ankley et al. 2003).

These and many more observations of wildlife demonstrate that global contamination of wildlife populations has dramatic effects on the health and reproductive potential of these populations. The phenotype we observe in individuals is produced by the environment acting on the genotype. The abnormalities we observe in wildlife are not due to classically held concepts of gene mutations. Instead, they represent alterations in the timing of gene expression and the level of gene expression. If exposure occurs during embryonic development, these alterations can be permanent (see Guillette et al. 1995). Wildlife have acted as sentinels for human health for centuries. An important issue is whether the abnormalities reported in wildlife provide a warning that human health and development are at risk.

Studies with Laboratory Animals

Numerous studies in laboratory animals have documented profound embryonic disruption by low level exposure to environmental chemicals including pesticides (herbicides, insecticides and fungicides) and those contained in a range of industrial products (e.g., phthalate additives in polyvinylchloride plastic and the monomer bisphenol A used in the manufacture of polycarbonate plastic) profoundly disrupt fetal development. Parmigiani and colleagues at the University of Parma administered the widely-used insecticide methoxychlor to pregnant mice. The offspring were examined for neurochemical changes in the dopaminergic system in the basal ganglia area of the brain. Neurons that use dopamine as a neurotransmitter (the dopaminergic neural system) are involved in the control of locomotor activity and exploration. The basal ganglia was studied because one of the major impacts of methoxychlor on behavior is to increase exploratory activity to novel stimuli

(Parmigiani et al. 1998, Palanza et al. 2002). This is also the area of the brain where degeneration occurs in Parkinson's disease, as well as associated changes in behavior. A change in behavior was associated, particularly in females, with a decrease in dopamine receptors in the basal ganglia. Males exposed to methoxychlor also showed an increase in territorial behavior, which is associated with aggressiveness. These findings show that permanent changes in brain function and behavior are associated with very low levels of exposure to this pesticide, levels previously considered to be completely safe (P. Palanza, F. Morellini and S. Parmigiani, unpublished observation).

Since 1997, a large number of peer-reviewed journal articles has been published showing that bisphenol A causes harm in animals at levels to which the average human is exposed. Bisphenol A is another chemical that, similar to methoxychlor, has the ability to bind to estrogen receptors and initiate cellular responses similar to those caused by estradiol. However, bisphenol A was incorrectly initially thought to only be a very weak estrogen-mimicking chemical. Instead, recent experiments have shown that at "low doses" that had previously been predicted to be safe based on models, not data, bisphenol A has dramatic adverse effects. Recent findings include chromosomal damage in developing oocytes in mouse ovaries, and abnormalities in the entire reproductive system in male mice, including a decrease in testicular sperm production and a decrease in fertility. In addition, fetal exposure to bisphenol A increases the rate of postnatal growth and decreases the age at which females mature sexually (go through puberty). These females also have mammary gland abnormalities and appear pre-cancerous by the time the females reach young adulthood. Bisphenol A also causes abnormal brain development, and changes in brain function and behavior, similar to methoxychlor.

Assessing risks posed by EDCs to human health

It is likely that EDCs pose a significant threat to human health that classical epidemiological methods may not have the sensitivity to detect. Human studies on EDCs, which fall under the broader heading of environmental epidemiology, share many features with studies of environmental exposures such as to radon or

total suspended particulates, which are not endocrine disruptors. However, they differ from non-EDC studies in several important ways (study hypothesis, exposure(s), effect(s), model selection, analysis and interpretation) that make detection of effects more difficult. We will consider these points and then examine a recent study that circumvents at least some of these problems.

What triggers an investigation between an EDC and a human health effect? Traditional ("classical") epidemiological studies were often designed to investigate unusual patterns of human health outcomes. Perhaps the most dramatic of these was the investigation of diethylstilbestrol (DES) in response to a cluster of seven cases of a rare vaginal cancer (clear cell adenocarcinoma) in young women. Similarly, an awareness of increasing rates of lung cancer triggered the first studies of smoking and lung cancer. Some epidemiological studies of EDCs have similar origins. Indeed, DES itself is a quintessential EDC, and current research into possible EDC involvement in breast cancer causation and fertility impairment have been provoked by observations of human trends.

Many epidemiological questions raised by EDCs have their origins, however, in observations of impacts on laboratory animals and wildlife. These include the possible role of EDCs in increases in hypospadias, the effects of phthalates on male fertility, and the impact of polybrominated diphenyl ethers on neurocognitive development. In each of these cases, and many more, pronounced laboratory and field effects provoke questions about human impacts based on animal observations.

All else being equal, the ability of an epidemiological study to identify the cause of an adverse outcome decreases as the prevalence of the outcome and the number of causal factors increase. For example, the identification of DES as the cause of clear cell vaginal adenocarcinoma in young women was relatively easy because vanishingly few cases of this rare cancer had ever been documented in this age group, and no other cause had ever (before or since) been identified. Conversely, causes of breast cancer are notoriously hard to find not only because it is a complex, multifactorial disease but because of its extremely high lifetime incidence (one in eight women). The metaphor of signal detection may be helpful in clarifying this point; high

background levels of a disease contribute to background “noise “ (as do alternative causes, errors in exposure identification and diagnoses) and make detection of the “signal” (the association under investigation) difficult to identify. Epidemiology handles diseases of low incidence, and strong associations well, but multifactorial diseases of high incidence only poorly.

Consider the following thought experiment (Figure 3). Imagine a population of 5,000

women with a significant but not unusual spontaneous miscarriage rate of 10%, normally distributed. In that hypothetical population one would expect 500 miscarriages, ± 42 . In this experiment, expose 1% of the women to a contaminant that increases the risk of that abortion, on average by X-fold, with X increasing from 1 (no effect on risk) to a 10-fold increase. Elevation in risk would have to be more than nine-fold before the signal of exposure-induced miscarriage rose above background noise.

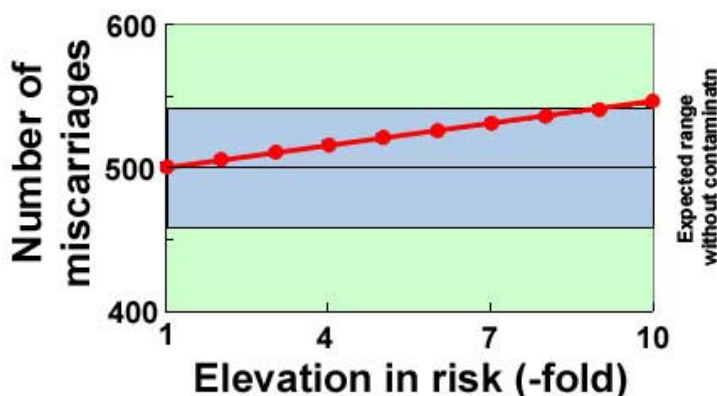


Figure 3. The expected number of miscarriages in a population of 5000 women as a function of risk elevated by exposure to a hypothetical contaminant. See text for parameters.

A crucial feature of EDCs is their “stealth” nature. Several recent studies have demonstrated that the general population has been exposed to, and currently carries measurable levels of, tens to hundreds of EDCs (CDC 2003, Thornton et al. 2003). The subject has no knowledge of these exposures; so the classical tools of epidemiologists (questionnaires, vital records, occupational histories etc.) provide no information. These presuppose the subject’s (or physician’s or employer’s) knowledge of exposure. Instead, it is necessary to obtain biological measures of exposure (biomarkers).

Biomarker studies require that subjects agree to provide a biological sample (e.g. blood, urine or saliva) and give permission for its use in such a study. Obtaining subjects willing to do this, Institutional Review Boards willing to approve these protocols, and funding for such studies is becoming increasingly challenging. An increasing number of studies are taking this approach; we describe a recent example below.

But for many EDCs, the analytical chemistry that would permit body burden measurements has not yet been developed, and for many for which it has, the chemical analyses are very costly, limiting sample size and thus statistical power. Moreover, the rapid metabolic degradation of some compounds means that single exposure measurements, for example from cord blood at birth, may completely miss critical exposures during pregnancy.

Whether distinguishing between exposure and non-exposure in cases and controls or estimating changes in risk as a function of increasing exposure, epidemiological studies traditionally assume monotonic dose response curves. Higher exposure levels are assumed to produce larger effects. Laboratory work with EDCs clearly shows, however, that non-monotonic curves are commonly found. The result of use of inappropriate models, such as those which assume monotonicity of dose response, and

absence of low dose effects, will result in “false negatives.”

Epidemiology regularly compares exposed and unexposed populations. Yet the global distribution of EDCs means that finding unexposed populations is virtually impossible. Classical epidemiological studies were designed primarily to examine isolated exposures, ignoring concurrent exposures or considering them as confounding factors to be treated as “nuisance variables.” This is inappropriate with EDCs, however, for two reasons. First, EDCs from similar and different chemical families can work through the same mechanism. They are thus substitutable. Unless the possibility of substitution is factored into the study by measuring multiple exposures and examining their joint risk, such mixtures will increase misclassification of exposure (an important source of conservative bias) and thus increase the likelihood of false negatives.

When mixtures of EDCs have been studied they have been seen to interact, often in unpredictable ways, with subadditive, additive and even synergistic effects. It is difficult, if not impossible, to isolate exposure to a single pesticide, phenol or phthalate. As the EWG-Mt. Sinai study showed (Thorntonn 2003), it is likely that all subjects are exposed to measurable amounts of large numbers of these chemicals, many of which act along common pathways. These factors pose a significant and currently unsolved challenge to epidemiology.

Long time lags between exposure and effect, which may span decades or even generations as in the case of DES, will further complicate detection of impacts. For nonpersistent compounds, all traces of the parent compound and its metabolites will likely have disappeared. With persistent contaminants, degradation of the parent compound into different metabolites, some toxic, some not, and some working via different mechanisms (e.g., DDT is estrogenic while its metabolite DDE is anti-androgenic) will further complicate interpretation even in cases where the study has measured biomarkers of exposure.

Aside from ecological studies, epidemiology is conducted at the individual level. Effects of classical exposures are usually binary outcomes in individuals, which are well defined and severe (cancer case vs. non-case, birth with limb

reduction or not). However, wildlife data suggests that changes from EDC exposure at the level of the individual are often subtle and difficult to classify (reduced fertility, poor semen quality, more feminine play behavior, genital dysmorphology). The effect of such changes at the population level, however, can be profound. As discussed above, trends in mean values of several outcomes have been reported but other changes at the population level, which may be even more profound, are increases in population variance and an increasingly non-normal (non-Gaussian) population distribution.

While EDCs manifestly present challenges to epidemiological studies, and are likely to have led to false negatives and underestimates of true risk, some progress is being made in developing approaches that acknowledge these pitfalls and employ methods explicitly designed to avoid them. One of us (Swan) has been involved in such a study, investigating reduced semen quality in relation to pesticide exposure. This study is somewhat unusual from an EDC perspective because it focuses on what appear to be adult-mediated impacts rather than developmental impacts. This avoids the problem of long time lags noted above.

A study of semen quality in relation to pesticide exposure

After finding that fertile men from the general population of an agrarian area (Columbia, MO) had decreased semen quality (for example, only 58% of the number of moving sperm as men from Minneapolis, MN) (Swan 2003A), pesticide exposure was examined as a cause of poor semen quality (Swan 2003B). The authors measured urinary metabolites of eight non-persistent, current-use pesticides in two groups of men from mid-Missouri; men with all semen parameters (concentration, % normal morphology and % motile) below median value (cases) and men in whom all semen parameters were within normal limits (controls). Pesticide metabolite levels were particularly elevated in cases compared to controls for the herbicides alachlor and atrazine, and for the insecticide diazinon (2-isopropoxy-4-methyl-pyrimidinol, or IMPY) (p-values for Wilcoxon rank test = 0.0007, 0.012, and 0.0004, for alachlor, atrazine and IMPY, respectively). Men with higher levels of alachlor or IMPY were significantly more likely to be cases than men with low levels (OR=30.0, 16.7 for alachlor and IMPY, respectively), as were men with atrazine over the

LOD (OR=11.3). The number of pesticides found in the urine at elevated levels was significantly related to the risk of poor semen quality (being a case rather than a control). These associations were seen in the general population, who were not occupationally exposed. The three pesticides most strongly associated with semen quality are among the five that have been measured most frequently in drinking water sources in the mid-West. These are not removed by routine water treatment. Therefore, drinking water is the most plausible route of exposure. These findings suggest that adult exposure to several widely-used pesticides via drinking water is a likely cause of the reduced semen quality seen in fertile men from mid-Missouri.

Subject responses to questions about home and occupational pesticide use were not related to semen quality, suggesting that the relevant pesticide exposure was unknown to the subject. Therefore, collection of urine samples and assays for pesticide metabolites in the subject's urine using highly sensitive GCMS were required to document exposure to the low levels of pesticides that were related to semen quality. In addition, effects were seen at the level of the individual, with likely more profound effects at the population level. The average decrease in sperm concentration in fertile men living in mid-Missouri relative to men living in Minneapolis, MO is 40 million sperm/ml. While the median sperm concentration for Missouri men (54 million/ml) was within normal limits, the sperm count for about 40% of these men fell below 40 million/ml, the point at which fertility declines significantly (Bonde et al. 1998).

Summary

In this paper we have outlined evidence from a diversity of sources indicating that a variety of manmade compounds can interfere with sexual and brain development, resulting in reduced fertility, altered brain function and behaviour in wildlife, laboratory animals and humans. Four summary points emerge:

- Contaminants at low levels can interfere with gene expression.
- Wildlife, laboratory animal and human effects are strongly concordant.
- The available data are not consistent with several key assumptions traditionally used to guide regulatory science and regulations.

- Traditional epidemiology will have great difficulty establishing causation of effects of these chemicals in humans.

Implications

The concordance of animal and human data, where the latter are available, indicates that when human data are not available health standards should be guided by animal research on a precautionary basis. It will be decades, at best, before epidemiological science is capable of thoroughly documenting the health impacts of even a small number of the contaminants to which humans are exposed daily.

Zichichi (1993) has pointed out that many decisions are made about technology without an adequate scientific basis on which to assess costs and benefits. Endocrine disruption clearly fits that model, with toxicological data on risk emerging decades after exposures began. The regulatory system can and should serve public health more effectively.

Many of the chemicals of concern were produced to improve human welfare and provide economic benefit (for example, to increase crop production or to protect food from metal in food cans). This new science, however, is now revealing many unexpected adverse consequences, resulting from the ability of very low levels of these compounds to interfere with gene activation. Most of the chemicals now implicated were subject to little if any rigorous testing. Many tested using criteria now known to miss important risks were found "safe" and allowed to enter the marketplace. Now we are discovering their "stealth" characteristics only long after widespread exposure has occurred.

Because of their "stealth" nature, we are currently unprepared to detect the effects of EDCs or defend against them. Many are persistent; they cannot be removed; they are globally distributed through our atmosphere, our seas and wildlife. Others, while not persistent, should be treated as persistent because of their chronic and ubiquitous use. They act at a population level and many have the potential to (individually or cumulatively) affect future generations, for example by decreasing fertility, feminizing males or reducing intelligence. All these endpoints have been produced in the laboratory and many have been observed in wildlife. New data—which must be confirmed by further study—suggest that comparable changes are being produced in human

populations as well. Precaution dictates that we cannot wait for “conclusive” evidence of harm to human populations to take action.

Chemical corporations and government agencies charged with regulating chemicals in the environment (air, soil, water and food), assure the public that these chemicals are safe. Because of absence of data concerning risk (often confused with evidence of an absence of risk) and the use of conservative models no longer supported by recent data, the public remains ignorant of the risk potential of the vast majority of chemicals. The public is routinely informed that these chemicals have been tested, that there are studies demonstrating the absence of their risk, and that regulatory agencies adequately protect public health.

Clearly significant changes are needed to bring current regulatory practices into conformity with new scientific information. We propose that testing for health effects at doses within the range of human exposure (currently not done) with respect to long-latency effects of developmental exposure throughout the lifespan (currently not done) be required prior to the introduction of any chemical intended for use in commerce.

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