Endocrine Disrupting Contaminants—Beyond the Dogma

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Descriptions of endocrine disruption have largely been associated with wildlife and driven by observations documenting estrogenic, androgenic, antiandrogenic, and antithyroid actions. These actions, in response to exposure to ecologically relevant concentrations of various environmental contaminants, have now been established in numerous vertebrate species. However, many potential mechanisms and endocrine actions have not been studied. For example, the DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] metabolite, *p,p´*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethyl-ene] is known to disrupt prostaglandin synthesis in the uterus of birds, providing part of the explanation for DDT-induced egg shell thinning. Few studies have examined prostaglandin synthesis as a target for endocrine disruption, yet these hormones are active in reproduction, immune responses, and cardiovascular physiology. Future studies must broaden the basic science approach to endocrine disruption, thereby expanding the mechanisms and endocrine end points examined. This goal should be accomplished even if the primary influence and funding continue to emphasize a narrower approach based on regulatory needs. Without this broader approach, research into endocrine disruption will become dominated by a narrow dogma, focusing on a few end points and mechanisms. *Key words:* adrenal steroidogenesis, endocrine disruption, estrogens, hormone metabolism, nitrate, progesterone, prostaglandins. *Environ Health Perspect* 114(suppl 1):9–12 (2006). doi:10.1289/ehp.8045 available via *http://dx.doi.org/* [Online 21 October 2005]

In 1991 a hypothesis was formulated to suggest that numerous xenobiotic chemicals used in everyday commerce or natural chemicals released into the environment by human activity had the potential to disrupt the endocrine system of wildlife and humans at ecologically relevant concentrations. This hypothesis has become known as the endocrine-disrupting contaminants (EDCs) hypothesis (Colborn and Clement 1992). Since the presentation of this original hypothesis more than a decade ago, significant scientific and public debates have raged concerning its validity. Much of the early work was driven by wildlife observations that documented *a*) estrogenic, androgenic, antiandrogenic, and antithyroid actions in fish found below outfalls of sewage or paper pulp mills; *b*) exposure of alligators to agricultural chemicals; and *c*) exposure of birds, fish, and mammals to complex mixtures of chemicals in the Laurentian Great Lakes of North America or the Baltic Sea of Northern Europe [for representative reviews, see Crain et al. (2000); Fox (1992); Guillette and Gunderson (2001); Tyler et al. (1998)]. A growing literature supports these early observations and has extended them, thus supporting the hypothesis that various chemicals alter endocrine function in a wide array of vertebrates and invertebrates (Gray et al. 2002; Iguchi et al. 2001; Markey et al. 2002; Oberdörster and Cheek 2000). The questions being addressed today are not whether endocrine disruption occurs because of contaminant exposure but rather at what concentrations does it occur? Or, what is the mechanism of action driving the response? Or, is the response observed adverse at the population level?

Major debates in the scientific and public arena continue to arise around the issues of EDC-induced human health effects, especially breast cancer, human semen quality, and birth defects of the genitalia and reproductive system (McLachlan 2001; National Academy of Sciences 1999). As with wildlife, a number of studies report an association between contaminant exposure and alterations in the development and functioning of the male reproductive system (Sharpe and Irvine 2004; Toppari et al. 1996). However, numerous studies also document the difficulty of establishing the link between exposure and health outcomes in human populations [for discussion, see Birnbaum and Fenton (2003); Carpenter et al. (2002); Harvey and Johnson (2002); Safe et al. (2002)]. Thus, many studies resort to examining mechanisms in model species or tissues and then relating this information to human populations (e.g., Mori et al. 2003). This approach was dramatically supported recently, when a study of baby boys revealed that end points used in rodent studies were useful in examining the developmental biology of humans. Swan et al. (2005) demonstrated that anogenital distance, a frequently used end point in rodent developmental toxicology studies, decreased significantly with increasing environmental exposure to phthalates, as reported in prenatally exposed rodents. In short, as we have learned from drug exposure studies, many end points in rodents can predict adverse outcomes in humans.

Estrogens and estrogenic actions have dominated the EDC literature since the early 1990s. The scientific literature associated with understanding the legacy of diethylstilbestrol (DES)

exposure during pregnancy has been a prominent model (Bern 1992). That is, the scientific community in collaboration with DES advocacy groups composed of mothers and exposed children has studied the effects of embryonic and neonatal exposure to a potent synthetic estrogen. These data, from affected human populations and laboratory rodent model systems, have provided much of the basic scientific literature for arguing adverse health effects in human populations exposed to EDCs (Markey et al. 2003; McLachlan 2001). This dependence on documented adverse human health effects and laboratory rodent models has led to a vast majority of EDC research focused on estrogenic actions, especially those associated with estrogen receptor–mediated actions. This work has been reinforced by wildlife studies using estrogenic end points such as yolk production in male fish (Sumpter and Jobling 1995). Additional studies that demonstrate the antandrogenic actions of *p,p´*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], the major bioaccumulated metabolite of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], and various fungicides that act on the androgen receptor (Gray et al. 2001) have supported the perception that EDC action primarily produces an "estrogenic" feminizing or demasculinizing response. Discussion of the antithyroidal actions of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) has broadened the research in EDCs, but current approaches focus almost exclusively on the neurological or neuroendocrine actions of these compounds in developing embryos (Rolland 2000; Zoeller et al. 2002). All these actions are worth study, but the focus on estrogenic, antiestrogenic,

and antithyroidal outcomes has produced a dogma that EDC research is largely about receptor binding or mechanisms that are largely based on sex steroid or thyroid action. From the perspective of regulatory science, a focus on one or two mechanisms is cost- and time-effective, and given the economic and regulatory issues surrounding EDC research, a "realistic" approach (Ankley et al. 1997; Kavlock et al. 1996). But from the perspective of advancing the science, this limited view has had a restrictive effect. Widening this view is essential if further advances in our understanding are to occur.

In this article, I suggest some areas where future research is needed to broaden our understanding of endocrine disruption by environmental contaminants. This article is by no means a review but rather a short outline of areas where recent data suggest that future work could produce insight into mechanisms of endocrine disruption.

Estrogens: Steroid Actions beyond Receptor Binding

Although many contaminant-induced estrogenic actions described to date appear to be caused by receptor-mediated interactions, other responses are less clear (McLachlan 2001). Several studies have provided support for the hypothesis that estrogenic actions could be due to increased aromatase activity. For example, the feminizing effect of atrazine on some species has been hypothesized to be caused by its ability to alter aromatase-induced conversion of androgens to estrogens (Crain et al. 1997; Hayes et al. 2003). Several studies with mammalian or fish cell lines suggest that such a mechanism is possible (Sanderson et al. 2000, 2001; Sanderson and van den Berg 2003). Future studies must consider a mechanism by which contaminants alter steroid action not by receptor binding but rather via alterations in the synthesis of these hormones by modification in the enzymatic pathways, either by direct changes in enzyme synthesis or in enzyme activity levels (i.e., alterations in available cofactors) [for discussion see Harvey and Johnson (2002); Sanderson and van den Berg (2003)].

Glucocorticoids and Progestins: Steroid Actions beyond Estrogens and Androgens

Before the presentation of the EDC hypothesis, a metabolite of DDT, o,p'-DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane], was known to disrupt adrenal steroidogenesis in some mammalian species, including humans (Benecke et al. 1991; Ruppert and Kraft 1999). In fact, this compound has been marketed as a drug to treat adrenal cancer and Cushing's disease because of its suppressive action on adrenal steroidogenesis (Benecke et al. 1991). Even with this knowledge, few investigators have examined wildlife species or humans for alterations in

adrenal function in areas where DDT use is still extensive, as in many tropical regions with a high risk of malaria. Not all species will respond to this compound with altered adrenal steroidogenesis (Breuner et al. 2000), but future studies on a wide array of species are needed.

Furthermore, because of the action of o,p'-DDD in humans, that it is a major metabolite of DDT, and that DDT is still used extensively in tropical regions, more studies are needed that examine human populations for adrenal-based end points, especially pregnant women. At least one recent study has suggested that coincident with the use of DDT and elevated DDE plasma levels, a significant increase in human preterm parturition occurred (Longnecker et al. 2001). It is now commonly accepted that the hypothalamo–pituitary–adrenal axis is central to the control of gestation length and the timing of birth in humans, and probably in eutherian mammals in general (Chrousos et al. 1998). Alterations in adrenal steroidogenesis and in glucocorticoid feedback to the hypothalamo–pituitary axis are associated with disrupted gestation and birth, which suggests that chemicals capable of acting on this system are potentially important in human reproductive health.

Progesterone and related progestins are another group of steroid hormones with a central role in gestation maintenance in every vertebrate species studied to date (Jones and Baxter 1991). These hormones are essential for oocyte maturation as well. Recent studies identified a receptor for these steroids in the cell membrane (Zhu et al. 2003a, 2003b), in addition to the traditional perinuclear receptor, which acts as a transcription factor (Carson-Jurica et al. 1990). Some species can have more than one form of the perinuclear receptor with unique tissue distributions (Custodia-Lora and Callard 2002). Very few studies have examined the action of environmental contaminants on progestin-based phenomena. Various contaminants can bind the nuclear progesterone receptor (aPR) from the American alligator (Vonier et al. 1996). Although many of the chemicals tested, including most of the DDT metabolites, did not reduce [³H]R5020 binding to aPR, 2,2-bis(p-chlorophenyl)ethanol (DDOH, a DDT metabolite), endosulfan, alachlor, dicofol, atrazine, and kepone did (Vonier et al. 1996). Pickford and Morris (1999) have demonstrated that methoxychlor disrupts progesterone-induced oocyte maturation in the frog *Xenopus leavis*. This action appears to be independent of the nuclear receptor. Progesterone plays a major role in gestation maintenance; thus, a disruption in progesterone signaling could also affect the frequency of spontaneous miscarriage, as described above. In short, we need to broaden our concepts of the mechanisms and end points used to assess steroid mimicry by environmental contaminants.

Prostaglandins and Uterine Functioning: the Past and the Future?

Dramatic declines in the populations of numerous bird species occurred during the decades following the wide-scale use of DDT for insect control in the United States and other countries around the world. Upon examination, DDE, the major metabolite of DDT was associated with altering the physiological process of eggshell formation that subsequently led to eggshell thinning and population declines of numerous avian species, particularly raptors and shorebirds [see review by Lundholm (1997)]. These studies indicate that the supply of calcium to the eggshell gland is not impeded by DDE, but rather this organochlorine contaminant disrupts calcium transport within the eggshell gland (Lundholm 1997). In addition, prostaglandins (PGs) have been implicated in eggshell thinning because DDE disrupts PG synthesis, which reduces bicarbonate transport in the duck shell gland lumen, thereby reducing calcium transport.

Given the important roles of various PGs during ovulation, pregnancy, and parturition in a wide array of vertebrate species (Guillette et al. 1991; Jenkin and Young 2004), it is surprising that few investigators have examined the disruption of this important class of hormones. PG synthesis, like steroidogenesis, is controlled by a pathway of enzymes (Helliwell et al. 2004a). Steroids, particularly estrogens, regulate the expression of a number of these enzymes. Thus, with a wide range of chemicals capable of altering the "estrogenic milieu," it could be hypothesized that PG synthesis is altered in the reproductive system of any vertebrate, as described previously for the avian reproductive tract. Interestingly, recent studies have suggested that PGs could work through perinuclear receptors as well as through the established membrane–G protein receptors (Helliwell et al. 2004b). If this is the case, disruption at the level of transcription could occur via several mechanisms leading to different outcomes. Finally, it is important to recognize that PGs are not just reproductive hormones. Rather, they play essential roles in the immune response, including inflammation and arthritis, cardiovascular regulation encompassing hypertension, and respiration including asthma. These are just some of the roles that ubiquitous PG hormones play in vertebrates. Moreover, PGs are evolutionarily ancient and are important in reproduction and development in invertebrates as well (Stanley and Howard 1998). In brief, a major research effort is needed to examine the possible linkages between the wide-ranging alterations seen in organisms living in contaminated environments and alterations in prostaglandin synthesis.

Hepatic Metabolism of Hormones: Changing the Hormonal Milieu

Many vertebrates exhibit species-specific, sexually dimorphic patterns of hepatic enzyme activity that appear to be regulated by sex steroids and/or growth hormone (Gustafsson 1994). In humans and rodents, 50% or more of the drugs and pesticides currently used induce the expression of the hepatic enzyme cytochrome P450 (CYP)3A (Gibson et al. 2002). Numerous steroids, including testosterone, 17β-estradiol, progesterone, and androstenedione, are metabolized by CYP3A (Gibson et al. 2002). It is therefore conceivable that wildlife or humans exposed to contaminated environments would exhibit elevated CYP3A activity that leads to increased clearance of such steroids as testosterone and 17β-estradiol from the plasma, as reported in chickens (Chen et al. 1993, 1994) and humans (Bammel et al. 1992).

Blumberg et al. (1998) first proposed the "steroid sensor hypothesis" by suggesting that the induction of hepatic enzymes responsible for the metabolism of endogenous and exogenous compounds was regulated by a nuclear receptor with broad specificity. A group of nuclear receptors (e.g., steroid xenobiotic receptor, SXR) was discovered, and this hypothesis has been further supported by studies that have observed a positive relationship between SXR expression and CYP3A expression (Blumberg and Evans 1998). Transcription of CYP3A, as well as of other hepatic enzymes involved in the biotransformation of testosterone, pharmaceutical agents, and xenobiotics, appears to be regulated, at least in part, by SXR and related nuclear transcription factors (Blumberg et al. 1998; Xie et al. 2000). Differences in the activity of the hepatic enzymes responsible for the biotransformation of sex steroids exist among alligators collected from contaminated and relatively uncontaminated sites in Florida (Gunderson et al. 2001, 2004). We have hypothesized that differences in plasma sex steroid concentrations could be caused, in part, by differences in hepatic clearance of these sex steroids, as has been demonstrated in other species (Wilson and LeBlanc 1998, 2000). Future studies need to consider the complex interactions among contaminant exposure, hepatic enzyme induction, and regulation of circulating hormone concentrations (e.g., Tabb et al. 2004) because these issues are not separate but are, in fact, tightly integrated.

It has been observed relatively recently that the promoter region of the human gene for inducible nitric oxide (NO) synthase (iNOS) has response elements for the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). These orphan receptors and transcription factors are related to SXR [see above and Toell et al. (2002)]. Specifically, PXR activation by xenobiotics appears to up-regulate iNOS and subsequently increases NO synthesis. Inflammation and apoptosis are phenomena regulated by NO, as are various actions of the hypothalamo–pituitary–gonad axis. Interestingly, xenobiotic effects along this axis could be explained by modulations in iNOS that lead to altered hormonal action of NO. However, this explanation is not the only possible source of elevated NO because nitrate pollutants could also alter homeostasis in NO synthesis (Guillette and Edwards 2005).

Novel Mechanisms of Endocrine Disruption

Nitrates: an endocrine disruptor? It appears that nitrate, a global pollutant of most aquatic systems, has the potential to be an endocrine-disrupting contaminant (Guillette and Edwards 2005). Nitrate (NO_3) and nitrite (NO_2) have known physiological influences (Jensen 2003; Levallois and Phaneuf 1994; Zraly et al. 1997). Nitrate and nitrite have been reported for decades to be toxic in humans and animals (Avery 1999). As early as 1945, methemoglobinemia was associated with drinking nitrate-contaminated well water on farms from the Midwest United States. Those concerns as well as new ones continue today with increasing contamination of groundwater with nitrates (Avery 1999; Porter et al. 1999). Nitrates have been implicated as a disruptor of gonadal steroidogenesis (Panesar 1999; Panesar and Chan 2000; Zraly et al. 1997) and thyroid function (Kursa et al. 2000; Lahti et al. 1985; Wyngaarden et al. 1952, 1953). A recent retrospective study of our work on alligators from various lakes in Florida suggests that nitrate could contribute to some of the altered endocrine parameters previously reported in juvenile animals (Guillette and Edwards 2005). Nitrate could alter steroidogenesis by *a*) conversion to nitrite and nitric oxide in the mitochondria (Meyer 1995; Nohl et al. 2001, 2000; Zweier et al. 1999), which is the site of initial steroid synthesis (Stocco and Clark 1996); *b*) altering Cl^- ion concentrations in the cell by substituting for Cl^- in the membrane transport pump (Alrefai et al. 2001; Panesar 1999); or *c*) binding to the heme region of various cytochrome P450 enzymes associated with steroidogenesis and altering enzymatic action (Danielson 2002; White et al. 1987). More research is needed to examine the possible endocrine disruptive action of this ubiquitous pollutant.

Novel research systems in endocrine disruption. Several exciting studies have expanded our understanding of systems capable of endocrine disruption, such as the studies documenting contaminant-induced disruption of the interspecies signaling system associated with the nitrogen fixation pathway (Fox et al. 2001), thyroid hormone–dependent gene expression during metamorphosis in amphibians (Crump et al. 2002) or pheromonal communication among salamanders (Park et al. 2001; Park and Propper 2002). These examples are just a few of the natural systems that require further research efforts. Although regulatory biology and most research funding will continue to focus on a few model systems and mechanisms, a broad basic research agenda is needed if we are to move the field forward. From my perspective, research in endocrine disruption represents less a new field, but rather more an integration of many fields, including biology, chemistry, epidemiology, and atmospheric and earth sciences. It is the new synthesis of science by which we will begin to understand the complexity of the world around us and our impact on it. New understanding of the effect of contamination has been gained because comparative endocrinologists, developmental biologists, ecologists and evolutionary biologists have exchanged ideas, knowledge, and hypotheses with physicians, epidemiologists, toxicologists and biochemists. Understanding the complex interactions between the plethora of environmental contaminants and the organisms living in contaminated environments will require many approaches. The most important is a broad, creative approach to the science and its interpretation.

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