Developmental Effects of Endocrine-Disrupting Chemicals in Wildlife and Humans

Thee Colborn, 1 Frederick S. vom Saal, 2 and Ana M. Soto 2

1W. Atten Jones Foundation and World Wildlife Fund, Washington, DC 20037 USA;
2Division of Biological Sciences and John M. Dalton Research Center, University of Missouri, Columbia, MO 65211 USA; 3Department of Anatomy and Cellular Biology, Tufts University, Boston, MA 02111 USA

Large numbers and large quantities of endocrine-disrupting chemicals have been released into the environment since World War II. Many of these chemicals can disrupt development of the endocrine system and of the organs that respond to endocrine signals in organisms indirectly exposed during perinatal and/or early postnatal life; effects of exposure during development are permanent and irreversible. The risk to the developing organism can also come from direct exposure of the offspring after birth or hatching. In addition, transgenerational exposure can result from the exposure of the mother to a chemical at any time throughout her life before producing offspring due to persistence of endocrine-disrupting chemicals in body fat, which is mobilized during egg laying or pregnancy and lactation. Mechanisms underlying the disruption of the development of vital systems, such as the endocrine, reproductive, and immune systems, are discussed with reference to wildlife, laboratory animals, and humans. Key words: developmental effects, endocrine disrupters, differentiation, endocrine function, estrogen, fertility, hormones, organization, pollution, reproduction, xenobiotics.

Environmental Health Perspectives 10: 378-384 (1995)

Convincing evidence exists that a variety of pollutants, some of which can disrupt endocrine development in wildlife and laboratory animals, is found in rain water, well water, lakes, and oceans, as well as in fish, shellfish, dairy products, and processed products. This paper identifies the need for a greater awareness about the long-term health consequences associated with exposure to endocrine-disrupting chemicals during early life. Endocrine-disrupting effects are not currently considered in assessing risks to humans, domestic animals, and wildlife. Taking into consideration what is currently known about chemicals that disrupt the endocrine system, the effects 1) may be manifested in an entirely different way, and with permanent consequences, in the early embryo, fetus, and neonate from effects as a result of exposure only in adulthood; 2) can change the course of development and potential of offspring, with the outcome depending on the specific developmental period(s) of exposure; and 3) are often delayed and thus may not be fully or obviously expressed until the offspring reaches maturity or even middle age, even though critical exposure occurred during early embryonic, fetal, or neonatal life.

In mammals as well as all other vertebrates, communication among sex-specific organs required for development to progress normally. Substances produced by one or more groups of cells can direct the course of development and thus determine the future functioning of another group of cells (5). For example, a group of compounds, the steroid hormones produced by the mother’s ovaries and adrenal glands, the placenta, and the fetal gonads and adrenal glands, has been identified as playing a major role in regulating developmental processes in many tissue (2). Organogenesis, a particularly vulnerable stage of development, begins in humans at the end of the second month of gestation. At this time the course of development of many tissues is regulated by endogenous steroid hormones along with other endocrine and paracrine factors (5).

It is now recognized that numerous endocrine-disrupting chemicals have been released into the environment in large quantities since World War II (Table 1). Some of these chemicals bind to intracellular receptors present for steroid hormones (4) and evoke hormonal effects in animals (5), humans (6), and cell culture (7,8). Thus, these chemicals produce biological effects whose normal role is to mediate the effects of the endogenous steroid hormones (9). Laboratory studies have demonstrated that exposure to endocrine-disrupting chemicals can profoundly disrupt differentiation of brain centers (10,11) because they can act as hormone agonists or antagonists. Organs that appear to be at particular risk for developmental abnormalities in offspring because of maternal exposure are those with receptors for gonadal hormones in female fetuses: this includes the mammary glands, fallopian tubes, uterus, cervix, vagina, and in male fetuses it includes the prostate, seminal vesicles, epididymides, and testes. In both sexes the central nervous system, brain, skeleton, thyroid, liver, kidney, and immune system are also targets for steroid hormone action and are thus potential targets for endocrine-disrupting chemicals, although these chemicals may have multiple modes of action, in addition to acting as hormone agonists and antagonists, in different target tissues (11-15).

A major concern is the profound and permanent effects that exposure to endocrine disrupters during critical periods in development can have on the future well-being of wildlife and humans, although chronic exposure after maturity can also present a health risk. It is generally assumed that after maturity, exposure to endocrine disrupters may only alter the functioning of hormone-responsive tissues. Studies in animals have shown permanent changes in brain (16) and vaginal epithelium (17) by females and a range of effects such as the administration of estrogenic chemicals in childhood. The possibility thus exists that chronic, low-level exposure to estrogenic chemicals in the environment after maturity can have effects in humans similar to those observed in laboratory animals administered estrogen (18).

Wildlife Exposure to endocrine-disrupting chemicals in the environment has been associated with abnormal thyroid function in birds (20) and fish (21), decreased fertility in birds (22), fish (23), shellfish (24), and mammals (25); decreased hatching success in fish (20, 27), and turttles (28); demasculinization and feminization of male fish (29), birds (30), and mammals (31); deformation and masculinization of female fish (32), gastropods (33), and birds (38); and alteration of immune function in birds (44) and mammals (45). These deleterious health effects have been observed in many areas where the presence of multiple endocrine-disrupting factors, such as byproducts of industrial chemical synthesis (chemical waste) and pesticides (39), has been established. The effects were not reported before the 1950s and are currently observed in many species of multiple taxa, especially birds (20) and mammals (31). It is important to note that the level of contamination in the Great Lakes region is no greater than some of the other major drainage basins in the United States (37).

Researchers from Guelph University report a 100% prevalence of thyroid enlargement in 2-4-year-old salmon in the Great Lakes. Moreover, in some Great Lakes salmon stocks, there is an extremely high prevalence of precocious sexual maturation in males (induced in and around the Great Lakes) and low egg thyroid hormone content (32). Multitoe abnormalities, including behavioral

Addres correspondence to T. Colborn, W. Atten Jones Foundation and World Wildlife Fund, 1219 25th Street NW, Washington, DC 20037 USA. Received 23 February 1995; accepted 2 July 1995. 378 Environmental Health Perspectives
changes, reproductive loss, and early mor-
tality in offspring have been documented
in bird species that feed on Great Lakes
fish (38). Reproductive loss and early mor-
tality have also been observed in offspring
of confined mink that fed Great Lakes
fish (39).

The devastating effect of DDT on
embryonic survival in bald eagles due to
eggshell thinning in Canada has been known
for some time (40). DDT was intro-
duced on a large scale into the envi-
ronment in the early 1940s. Restrictions
on the use of DDT since 1973 have been
very partially successful in reducing levels
in the Great Lakes (36). Monitoring nest-
ing sites along the Great Lakes shoreline
indicates that while eggshell thinning has
abated, embryonic and chick survival is not
adequate to maintain stable populations.
Restrictions are from a suite of chemicals
that responded to the restriction of DDT
and other chemicals and that do not
depend on contaminated fish in the Great
Lakes as a primary food source. However,
adult bald eagles that migrate to the shore-
line have difficulty producing viable off-
spring after consuming fish and other food
from the Great Lakes for 2 or more years
(38). The shoreline has thus become a
"black hole" for bald eagles that migrate to
these areas for food. It is not known how
much of the decline in reproductive success
has occurred because of the DDT (41).

Results from several studies indicate
that bald eagles may also be affected by
other contaminants, such as polychlorinated
biphenyls (PCBs) (42). DDT, PCBs, and
other synthetic organic compounds are
found in the eggs of many fish species,
and their presence has been implicated in
reproductive failure. DDT and PCBs are
both known to cause eggshell thinning in
eagles (43). Studies have shown that
DDT and PCBs can be transferred through
the food chain and have been detected in
the eggs of bald eagles (44).

Dieldrin is also a concern because
it is a dermonecrotic agent. It was first used
in the early 1940s as a pesticide and is still
used today. Dieldrin is highly toxic to fish
and other aquatic animals, and its effects
on reproduction and development have been
studied extensively. Dieldrin can cause
abnormal development and death of
embryos and larvae in a variety of species,
including birds, fish, and mammals (45).

The effects of DDT and PCBs on the
reproductive system are well documented
in many species. DDT has been shown to
cause a decrease in eggshell thickness and
result in a higher incidence of deformed
eggs. PCBs have been implicated in
reproductive failure due to their ability to
interfere with the production of thyroid
hormones and disrupt the endocrine system.

DDT and PCBs can accumulate in
the body fat of birds and are stored for
long periods of time. These chemicals can
be passed from one generation to the next
through the eggshell, and the effects can be
passed on to future generations. The
incidence of reproductive failure in bald
eagles has been linked to the presence of
these chemicals in the eggs (46).

DDT and PCBs are also known to affect
the development of the nervous system
and cause behavioral changes. These effects
are often seen in birds that have been
exposed to high levels of these chemicals
in their eggs (47). The effects of DDT and
PCBs on reproduction and development
are well documented, and they are some of
the most significant environmental
contaminants currently affecting wildlife.
There is a substantial literature documenting the detrimental effects of exposure to DES during the critical period of organ differentiation in experimental studies using rodents. Antifetal models capable of clinical studies in humans. For example, dysplastic changes in the vaginal prostate, (55) are comparable to those seen in stillborn male offspring of women treated with DES (56). In female mice, DES exposure during early life leads to permanent commi-
cication of the vaginal epithelium, which might be a factor in the brain-pituitary-gonadal axis (35,55,56). Significant implication of immune func-
tion, particularly to the T-cell system, has been also reported after exposure to DES during early life (57) as well as an increase in autoimmune diseases in women (58). These outcomes were typically not notice-
able at birth and often not detected before maturity. For example, treatment of male rats with DES during the first month after birth (accessory reproductive organs are still developing) (2) did not result in observable malignancies at 6-9 months of age, but by 20 months (old age), squamous cell cancer was detected with involvement of the dorsolateral prostate (59). In female mice treated during early life with DES, an increase in sensitivity of mammary glands to oncogenesis has been observed (60). A variety of agricultural and industrial chemicals produced today (either within or outside regulatory control) bind to intracellular estrogen receptors either directly, such as 4-endo-D,6-endo-D, or alteratively, through endogenous metabolites. For example, the pesticide methoxy-
chlorophyll (62) is demethoxylated in vivo to a more estrogenic biphenolic compound (63). Pesticides such as 4-endo-D,6-endo-D, chlo-
rene (64) and a number of other substances such as nonylphenol (7), mimic the action of endogenous estrogens and endogenous estrogens and synthetic estrogens (65). It is well known that estrogens are synthesized in endogenous mutants in culture (66). A number of conditions in wildlife are considered parallel to those observed in lab animals and humans exposed to endocrine-disrupting chemicals (1). It is worth noting that the exposure of chlorophenols was first detected in people working at a 4-endo-D,6-endo-D-producing plant (67), and although many effects of gen-
eric chemicals may be primarily due to exposure during in utero development, chronic exposure throughout adulthood is also a concern. For example, in studies with male dogs, which show spontaneous hyperplasia during aging, the disease only develops when exposed to 4-endo-D,6-endo-D, 51 with both estrogen and androgen, not androgen alone (58). Exposure of adult men to estrogen has been implicated in the etiology of prostate hyperplasia (15,65). Both prostate cancer and benign prostatic hyperplasia in men and cancer of estrogen responsive tissues in women (vaginal, cervical, endome-
trial, and breast) represent major medical problems faced by the female patient. It is now suspected that increases in the incidence of numerous pathologies in men and women may be related to exposure to pesticides and other endocrine-disrupting chemicals that can mimic DES and are therefore estrogenic. The clinical and experimental findings with DES show that the condition is an increase in breast and pro-
cstatic cancer in the United States occurred before 1970 and increased in incidence after 1970 (67). Although the trend of increase in prostatic hyperplasia in the United States between 1970 and 1987 (58,68,69), and an approximate 50% decrease in serum prostate worldwide over the last 50 years (70). These trends may be a reflection of the increase from estrogenic pollutants in the environment. It has been suggested that the decrease in serum of men is the result of exposure during the fetal peri-
od of tissue differentiation to pollutants that have estrogenic activity (71). For example, an association between reduced sperm motility and PCBs in mice with fer-
tility problems has been reported (72). Some PCBs are directly estrogenic while others become estrogenic after in vivo con-
version, although the binding affinity of estrogen receptors for PCBs is lower than that for DES (73). Characteristics of Endocrine- Disrupting Chemicals Literally thousands of synthetic com-
 pounds, a number of which are classified as endocrine-disrupting chemicals, have been released in the envi-
ronment, generating concern about their adverse and synergistic effects (74). Of particular concern are endocrine-disrupting chemicals that interfere with the action of hormones. It is common to find PCBs, dioxins, DDT, and a number of other pollutants in the environment, either in water or fish. It is necessary to assess the risk of exposure to these chemicals and other endocrine-disrupting chemicals. To do this, it is important to consider different times in life. For example, possible exposure to multiple estrogenic chemicals may be related to the fact that not all off-
spring of DES-exposed mothers show abnormalities. Although genetic factors may partially account for this outcome, it is also possible that the most affected indi-
viduals are those whose mothers were exposed to endocrine-disrupting environ-
mental pollutants with estrogenic activity before or during treatment with DES. Many of the effects of endocrine disruptors that have been reported in wildlife are associated with the presence of a toxic con-
comitant in the mother due to exposure before egg production in birds and fish or pregnancy and lactation in mammals. Evidence already exists that a number of organ systems become damaged or start to grow in the in, PBs, and DDT) has reached concen-
trations in aquatic food sources that can provide direct and indirect effects on the reproduction of fish (75). In addition, offspring of women who are more than twice as likely to have fish a fish a month for at least 6 years preceding their pregnancies were slightly preterm, had lower birth weight, smaller skull circum-
ference, and cognitive, motor (hypoactivity and hypoactivity and hypoactivity, and behavioral defi-
cits at birth compared with offspring whose mothers did not eat fish (76). The effects were associated with the mothers' lifetime experience of eating fish, not just what they are doing during pregnancy. These findings emphasize the importance of exposure of females to contaminants before pregnancy in terms of effects on their off-
spring. Subsequent studies of the above cut-off beginning at 6-7 months revealed declines in psychomotor development and poorer visual recognition compared with controls (77). In addition, the children of women who had eaten fish in this study exhibited short-term memory impairments (78). It is important to note that both phenotypic growth retardation and neurological effects which were related in a dose-dependent manner to the developmental sensitivity of the maternal food in fish (79). It remains to be determined whether the neurocognitive effects mentioned above are mediated through the endocrine system. It is recognized that the endocrine disrupting chemicals may act via multiple mecha-
nisms, some of which may only operate during specific developmental periods (11,80,81).
Based on current breast milk concentrations nationwide, it is estimated that at least 5% and possibly more of the babies born in the United States are exposed to quantities of PCBs sufficient to cause neurotoxic effects (48). This finding provides evidence that contemporary PCB exposure is above "any regulatory guideline," (20: 267). The purity of neurobehavioral and endocrinological consequences remain to be determined in these cohorts. A major concern is that some of these consequences may not become apparent until young adulthood or even middle age.

Accumulation of polychlorines increases the probability of repeated or constant exposure but, in the aggregate on diestin shows, administration at only one time in development, rather than the more likely chronic exposures, can result in developmental delay, the embryo, fetus, or perinatal infant. Ample evidence exists from both in vivo and in vitro studies of diestin can antagonize the action of estrogen in some estrogen target cells (10:46:10), although this effect does not appear to be due to estrogen binding to estrogen receptors (17). The fact that diestin is antiestrogenic is important because the conversion of androgen to estrogen in some target cells plays a critical role in masculinization (12). For example, a series of studies detailing the dose-related inhibition of hCG-induced masculinization of male mice has been reported in ovariectomy (17). This is particularly important during pregnancy when the concentration of estrogen-binding plasma proteins increases dramatically (2:3:9). It is possible that estrogenic chemicals may show low or no binding affinity to estrogen-binding plasma proteins. These chemicals may be able to freely enter cells similar to that in vivo, which would greatly increase their biological activity relative to similar blood concentrations of endogenous estrogen, most of which is eliminated from entering cells. This would contribute to the in vivo effectiveness of these polychlorinated hydrocarbons, which shows lower binding affinity to estrogen receptors than the most potent endogenous estrogens, estradiol (2:9). As a result, only a small fraction of the total endogenous estrogens in blood is able to pass into cells.

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Summary

The deleterious effects of estrogen-disrupting chemicals in the environment on the reproductive success of wildlife populations have been documented; this is not an isolated problem, and today many wildlife populations are at risk. At present, no coherent policy has been articulated to address this problem. This is due in part to the lack of knowledge concerning which of the many chemicals present in the environment are responsible for estrogen-disrupting effects. Regulatory agencies should recognize that the current endpoints of most tests to assess the risk of estrogens in other pollutants (carcinogenicity, acute toxicity, and immediate estrogenicity) have led to the misconception that these chemicals do not pose a threat to the health of wildlife, domestic animals, or humans. Although the effects of estrogens can be seen immediately in terms of gross abnormalities, the consequences of fetal exposure to endocrine-disrupting chemicals would likely not be recognized until several years after birth. Therefore, endocrine-disrupting chemicals are in most cases neither mutagenic nor acutely toxic at ambient concentrations. They may be released without concern into the environment. This may be partially remedied by screening for hormonally active and antagonistic activity using hormone-responsive cells in culture; this procedure identifies compounds that are estrogenic disruptors because they are hormonally active (40). Although this procedure cannot rule out chemicals that disrupt hormonal activity that may disrupt development through other mechanisms, it can at least rule out compounds like DDT, chlordecone, alkylphenols, and some PCBs, which are estrogenic agonists. It is also essential to continue to examine transgenerational effects in animal studies because some pollutants require metabolites in vivo to exert hormonal effects and because neurobehavioral and other developmental effects cannot be addressed in in vivo models (46:9:7).

Wildlife species have provided the model for maternal transfer of environmental endocrine-disrupting chemicals. The current concern is that these species may be less able to transfer these chemicals to their offspring since the mother's exposure is lower than that of the offspring. This transfer may be reduced by the body weight to the transfer of estrogenic activity from the embryo, fetus, or perinatal infant. Ample evidence exists from both in vivo and in vitro studies of diestin can antagonize the action of estrogen in some estrogen target cells (10:46:10), although this effect does not appear to be due to estrogen binding to estrogen receptors (17). The fact that diestin is antiestrogenic is important because the conversion of androgen to estrogen in some target cells plays a critical role in masculinization (12). For example, a series of studies detailing the dose-related inhibition of hCG-induced masculinization of male mice has been reported in ovariectomy (17). This is particularly important during pregnancy when the concentration of estrogen-binding plasma proteins increases dramatically (2:3:9). It is possible that estrogenic chemicals may show low or no binding affinity to estrogen-binding plasma proteins. These chemicals may be able to freely enter cells similar to that in vivo, which would greatly increase their biological activity relative to similar blood concentrations of endogenous estrogen, most of which is eliminated from entering cells. This would contribute to the in vivo effectiveness of these polychlorinated hydrocarbons, which shows lower binding affinity to estrogen receptors than the most potent endogenous estrogens, estradiol (2:9). As a result, only a small fraction of the total endogenous estrogens in blood is able to pass into cells.

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Volume 101, Number 9, October 1983

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