Endocrine-Disrupting Chemicals Use Distinct Mechanisms of Action to Modulate Endocrine System Function

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The term endocrine-disrupting chemicals is used to define a structurally diverse class of synthetic and natural compounds that possess the ability to alter various components of the endocrine system and potentially induce adverse health effects in exposed individuals and populations. Research on these compounds has revealed that they use a variety of both nuclear receptor-mediated and non-receptor-mediated mechanisms to modulate different components of the endocrine system. This review will describe in vitro and in vivo studies that highlight the spectrum of unique mechanisms of action and biological effects of four endocrine-disrupting chemicals—diethylstilbestrol, genistein, di(n-butyl)phthalate, and methoxyacetic acid—to illustrate the diverse and complex nature of this class of compounds. (Endocrinology 147: S25–S32, 2006)

A VARIETY OF structurally diverse natural and synthetic chemicals, classified as endocrine-disrupting chemicals (EDCs), have been reported to interfere with the endocrine system and ultimately disturb the normal function of tissues and organs, particularly those of the reproductive tract. Given their physicochemical differences and distinct biological effects, it is not surprising that a variety of mechanisms are used by EDCs to influence the endocrine system. Advances in our understanding of these mechanisms have been aided by increased public interest in the health effects of EDCs and the development of new tools and models for studying these compounds. Diethylstilbestrol (DES), genistein (Gen), di(n-butyl) phthalate (DBP), and methoxyacetic acid (MAA) are four compounds (Fig. 1) that are discussed here in an effort to illustrate some of the unique mechanisms of action used by EDCs to modulate endocrine system function.

DES

DES is a nonsteroidal synthetic estrogen that was developed by Sir Charles Dodds and colleagues in 1938 (1). Physicians began prescribing DES in the late 1940s to maintain normal placental steroid synthesis and prevent miscarriages and premature births (2). The results of the first randomized controlled clinical trials on the effectiveness of DES in preventing miscarriage and premature birth, published in 1953, showed no protective effect of DES (3). Despite these findings, DES continued to be prescribed to pregnant women until 1971, and it has been estimated that approximately 5–10 million Americans were treated with DES during pregnancy or exposed in utero from the 1940s to 1971 (4). Its use was discontinued in 1971 after a report that associated in utero DES exposure with vaginal clear cell adenocarcinoma, a rare form of reproductive tract cancer, in a small number (~0.1%) of daughters of women who had taken the drug (5, 6). Subsequent studies have reported multiple teratogenic effects attributable to prenatal DES exposure that occur more frequently than clear cell adenocarcinomas. In females exposed to DES in utero, nonneoplastic abnormalities such as anatomical malformations of the cervix, vagina, and uterus have been reported, as well as decreased fertility and less successful pregnancies (7–10). Although no increased risk of cancer has been observed in DES-exposed males, several teratogenic effects have been reported in the reproductive tract, including testicular hypoplasia, cryptorchidism, and epididymal cysts (7, 8, 11).

Adverse effects resulting from DES exposure continue to be uncovered as those exposed in utero advance in age. In an effort to better predict, prevent, and understand the effects of in utero DES exposure, several rodent models have been created to elucidate the mechanisms by which DES may impart its carcinogenic and teratogenic effects on humans. One well characterized DES exposure model is the neonatal mouse, in which female and male pups are treated with DES (2 µg/d) for the first 5 d of life and aged up to 18 months. Neonatal exposure of female mice to DES results in few reproductive tract abnormalities but a high incidence of benign reproductive tract tumors (12, 13). Although rodent models have effectively reproduced several elements of human DES exposure in utero, the complex developmental and carcinogenic effects of DES have made it difficult to study the mechanisms underlying DES-mediated action (13).

A variety of receptor-mediated and non-receptor-mediated mechanisms for DES-induced toxicity have been put
forward. The generation of mice lacking either estrogen receptor α (ERα) (14) or ERβ (15), i.e. ER knockout (ERKO) mice, provided effective tools to incorporate into the neonatal mouse model as a means to determine the role of the estrogen receptor (ER) in mediating DES-induced effects in vivo. Additionally, because there are two ER proteins, ERα and ERβ, the different animal models can be used to identify the role of each receptor, if any, in DES-mediated toxicity in specific tissues.

**Actions on females**

Studies in our laboratory have used the neonatal mouse model to study DES effects in female wild-type (WT), αERKO, and βERKO mice. WT females treated neonatally with DES displayed characteristic DES-induced reproductive tract lesions in the uterus, vagina, and oviduct (16) (Table 1). In the uterus, DES treatment produced atrophy, smooth muscle disorganization, hyalinization, squamous metaplasia of the luminal and glandular epithelium, and endometrial hyperplasia. In the vagina, DES treatment produced persistent epithelial cornification and vaginal adenosis in a small percentage of animals. Finally, in the oviduct, DES induced progressive proliferative lesions of the epithelium (16, 17). Similarly treated αERKO females displayed none of these characteristic DES-induced lesions, indicating that DES elicits its effects in the female reproductive tract through an ERα-dependent signaling pathway.

**Actions on males**

Similar experiments were performed in males to determine whether DES-mediated effects in the male reproductive tract, namely within the seminal vesicles and prostate, were ER mediated. DES treatment of WT males resulted in a significant decrease in seminal vesicle size at all ages, whereas no differences in the seminal vesicle sizes of DES- and oil-treated αERKO males were observed regardless of age (18) (Fig. 2). With respect to the prostate gland, histological analyses revealed that neonatal DES exposure in WT and βERKO mice resulted in changes in the aged ventral and dorsolateral prostates, including increased stromal mass, epithelial hyperplasia and dysplasia, and interstitial lymphocyte infiltration, all of which are characteristic of the phenotype of an estrogenized prostate gland (19). In addition, DES-induced epithelial cell differentiation defects indicative of estrogen imprinting were observed in the dorsolateral prostate of WT and βERKO mice, including the presence of a continuous layer of basal cells lining the epithelial ducts and acini, luminal cell hyperplasia, and loss of expression of dorsolateral prostate-2 protein, a marker of functional differentiation in the dorsolateral prostate (18). None of these effects were observed in the prostates of αERKO males treated with DES, indicating that ERα is required for DES-mediated effects in the prostate. Collectively, these data suggest that DES acts through an ERα-mediated mechanism in the male reproductive tract.

**Mechanisms of action**

Additional studies used these animal models to determine whether DES acts through an ERα-mediated mechanism to

![Fig. 1. The chemical structures of selected EDCs. The structure of 17β-estradiol is also shown for comparison.](image-url)
disrupt the expression of genes that are required for normal differentiation and organization of the reproductive tract. The Hox and Wnt families of genes represent potential targets for DES-mediated effects because their expression in the paramesonephric duct is critical for the development and organization of the female reproductive tract, and the reproductive tract phenotypes of null mouse models of the Hox and Wnt gene families are similar to those found in DES-exposed mice (20–24). Furthermore, neonatal DES exposure reduces the expression of Hoxa9, Hoxa10, Hoxa11, and Wnt7a in the murine female reproductive tract (23, 25, 26), further suggesting a role of altered Hox and Wnt gene expression in the observed reproductive tract phenotypes of DES-exposed mice. Based on these observations, experiments were performed in neonatal αERKO female mice to determine the role of ERα in mediating DES-induced down-regulation of Hox and Wnt gene expression. Expression of uterine Hoxa10 and Hoxa11 was reduced approximately 50%, and Wnt7a expression was reduced over 80% in the uteri of 5-d-old WT mice neonatally exposed to DES (16). In contrast to WT mice, DES had no effect on Hoxa10, Hoxa11, or Wnt7a expression in similarly treated αERKO mice, indicating that the DES-mediated reduction of the expression of these genes is regulated through ERα (Fig. 3). These results implicate an early developmental role for ERα in DES-induced teratogenic effects and suggest that disruption of Hox and Wnt gene expression may be ultimately responsible for the anatomical abnormalities in DES-exposed mice.

Subsequent studies have attempted to characterize the molecular mechanism underlying DES-mediated decreases in Hox and Wnt gene expression. One potential mechanism involves epigenetic modifications such as DNA methylation. For example, neonatal DES exposure increases lactoferrin and c-fos gene expression in the mouse uterus through demethylation of the lactoferrin promoter (27) and hypomethylation of exon 4 of the c-fos gene (28). However, DES treatment had no effect on the methylation state of the Hoxa10 and Hoxa11 proximal promoters in the mouse uterus, indicating that DES uses a different mechanism to modulate the expression of these genes (29). Additional studies have since identified a nonconsensus estrogen response element (ERE) in the Hoxa10 promoter that is differentially regulated by estradiol and DES (30). In these studies, the maximal
estradiol-induced luciferase expression of a Hoxa10-ERE-containing reporter plasmid was four times greater than the maximum induced by DES, indicating that the Hoxa10 ERE is induced in a ligand-specific manner, presumably because of the distinct conformational changes in the ER induced by ligand differences and by the Hoxa10 ERE. This molecular mechanism may account for the decreased Hoxa10 gene expression in the female reproductive tract after DES exposure and ultimately the distinct reproductive tract phenotypes observed in DES-exposed females. Whether a similar mechanism is responsible for the DES-mediated decreases in Hoxa11 and Wnt7a gene expression remains to be determined.

Gen

Several nonsteroidal plant-derived compounds known as phytoestrogens signal through the ER and therefore may act as endocrine disruptors. The potential benefits of phytoestrogens in preventing hormone-dependent cancers and lowering cholesterol have raised interest in studying the biological effects of these compounds. One such compound is Gen, an isoflavone present at high concentrations in soy-based products, which exhibits in vitro and in vivo estrogenic activity (31–33) as well as tyrosine kinase inhibitory properties (34). Although convincing evidence for both the beneficial and detrimental effects of Gen exposure is limited, it has been reported to reduce mammary cancer in rats (35, 36) and to lower cholesterol levels in humans (37, 38). However, it has also been associated with diminished reproductive capacity in animals (39, 40) and has been shown to induce uterine adenocarcinomas in a neonatal mouse model (41) and to increase the incidence of mammary tumors in rats (42). Further interest in studying Gen has resulted from the observation that humans, particularly infants, are exposed to it through their diet. It has been estimated that adults consuming modest amounts of soy-containing foods have a total isoflavone intake of approximately 1 mg/kg-d, whereas infants fed soy formula ingest significantly higher amounts, consuming 6–9 mg/kg-d of isoflavones, approximately 65% of which is Gen (43). This dose of isoflavones in infants is six to 11 times higher than the amount reported to have hormonal effects that alter the menstrual cycle in adult women (44).

Actions on the uterus

Given the ubiquitous nature of isoflavones in the human diet and the potential for both beneficial and adverse health consequences after exposure to Gen, efforts have been made to characterize the mechanism of action of Gen and to more clearly define the health effects of Gen exposure. In an immature mouse model, Gen elicits classical estrogenic uterotropic responses including increases in uterine wet weight, epithelial cell height, gland number, and lactoferrin expression (45). Receptor-binding assays indicate that Gen preferentially binds to ERβ in comparison with ERα, with relative binding affinities reported from 20- to 30-fold higher for ERβ (46, 47). Despite these differences in ligand binding affinity, Gen has only a slight preference for transactivation of gene expression in vitro through ERβ compared with ERα, suggesting that Gen may elicit effects in vivo through both ERα- and ERβ-mediated pathways (47). Evidence for ERα-mediated actions of Gen in vivo is derived from studies showing Gen-induced effects in the mouse uterus, which predominantly expresses ERα (45). In addition, Gen activates the IGF-I signaling pathway in the mouse uterus via an ERα-dependent mechanism (48). Taken together, these data suggest Gen acts through an ERα-mediated mechanism in the uterus.

Evidence suggesting an ERα-mediated mechanism for Gen action in the uterus has prompted studies in our laboratory to definitively establish the role of ERα in the uterine response to Gen. In these studies, ovariectomized WT and αERKO mice were treated with corn oil, estradiol (10 μg/kg-d), or Gen (50 μg/kg-d) for 3 d, and uterine weights were determined. Both the estradiol and Gen treatments significantly increased uterine wet weights in WT mice, whereas neither ligand increased uterine wet weights in the αERKO mice, providing direct evidence that ERα is necessary for Gen-induced uterotropic effects (unpublished data).

Actions on the ovary

To further determine the contributions of ERα and ERβ to Gen-induced effects in tissues other than the uterus, studies using ER null mice were incorporated into the neonatal mouse model described earlier, and the effects of Gen on the ovary were determined. Initial studies in WT CD-1 mice treated with Gen for 5 d showed a dose-dependent increase in multioocyte follicles by 19 d (49) (Table 2). To prove that the mechanism was due to the estrogenic properties of Gen and not to tyrosine kinase inhibitory properties, the nonestrogenic tyrosine kinase inhibitor lavendustin was incorporated into these experiments. No multioocyte follicles were observed after treatment with lavendustin regardless of dose, indicating that the increased incidence of multioocyte follicles was due to the estrogenic properties of Gen. Similar experiments were performed in C57BL/6 mice and in both αERKO and βERKO mice to determine whether Gen was signaling in the ovary through ERα, ERβ, or a non-receptor-mediated mechanism. Neonatal treatment with Gen increased the incidence of multioocyte follicles in WT C57BL/6 and αERKO mice in a dose-dependent manner. In contrast, βERKO mice treated with Gen had a significant decrease in the incidence of multioocyte follicles, suggesting that the

<table>
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<tr>
<th>Genotype</th>
<th>Genistein (μg/pup·d)</th>
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<tr>
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<td>Vehicle 1 10 100</td>
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<tr>
<td>αERKO</td>
<td>1/3 (1) 2/4 (1) 4/6 (4)</td>
<td>ND ND</td>
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<tr>
<td>βERKO</td>
<td>1/2 (1) 0/4 (0) 0/5 (0) 1/3 (2)</td>
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Data represent the number of mice that demonstrated at least one multioocyte follicle in any section examined. Numbers in parentheses are the highest number of follicles observed in a single section from that treatment group. ND, Not determined. [Reproduced from Ref. 49.]
induction of multioocyte follicles after neonatal Gen exposure requires ERβ (49) (Table 2).

Taken together, studies focused on the mouse uterus and ovary show that Gen is capable of signaling in a tissue-specific manner through both ERα and ERβ-mediated mechanisms. Although a variety of physiological and toxicological effects of Gen exposure have been illustrated in animal studies, conflicting data reported in epidemiological studies make it difficult to correlate animal models with human exposure (50). Additional studies characterizing other possible mechanisms of Gen action, such as non-receptor mediated, epigenetic, and transgenerational effects, are needed to broaden our understanding of the potential beneficial and detrimental effects of human exposure and to clarify inconsistencies between epidemiological studies.

**DBP**

Phthalate esters are used extensively as plasticizers and stabilizers in a variety of plastics and consumer goods. Exposure to phthalates through ingestion, inhalation, and dermal absorption occurs throughout life (51). Select phthalate esters, including DBP, adversely affect the male rat reproductive tract after either prenatal or postnatal exposure. These adverse reproductive tract effects, which include disrupted epididymal development, hypospadias, cryptorchidism, multinucleated gonocytes, and reduced fertility, are a result of the antiandrogenic effects of some phthalate esters (52). Interestingly, the reproductive tract abnormalities present in DBP-exposed rats are similar to those that occur in humans with testicular dysgenesis syndrome, which is believed to result from altered fetal development as a result of genetic mutations and/or pharmacological or environmental disruptions (53). Given the widespread use of phthalate esters, a potential role for DBP in testicular dysgenesis is plausible (51, 55). Humans are exposed to more DBP than any other phthalate ester, with maximal DBP exposure reaching 113 μg/kg/d (56, 57). Interestingly, these same studies showed that women of childbearing age have the highest estimated DBP exposures. However, these levels are considerably lower than the minimal reported dose of DBP necessary to alter male reproductive tract development of more than 50 mg/kg/d (52).

**Mechanisms of action**

Animal models in which rats are exposed gestationally to various phthalate esters have been used to characterize the endocrine-disrupting effects elicited by the phthalate esters. In these studies, DBP elicits its antiandrogenic effects by reducing testosterone production in the Leydig cells of the testis through several mechanisms. However, the androgen receptor (AR) antagonist fluoramide, in contrast to DBP, has little effect on the developing epididymis, suggesting a complex mechanism underlying DBP-mediated reproductive tract effects that involves more than its antiandrogenic properties (52). Furthermore, neither DBP nor its major metabolite, monobutyl phthalate, physically interacts with the AR, indicating that the antiandrogenic effects of DBP occur through AR-independent mechanisms (52).

One such established mechanism is the transcriptional down-regulation of genes associated with cholesterol transport (Scarb1 and Star) and testosterone biosynthesis (Cyp11a1, Hsd3b1, and Cyp17a1) that results in decreased testosterone production by the Leydig cells of the testis (58–60). Recent studies using microarray analyses to characterize the effects of DBP treatment on global gene expression profiles in the fetal rat testis show that DBP affects the expression of nearly 400 genes in Leydig cells, Sertoli cells, and gonocytes (61). With respect to the fetal Leydig cell, DBP increased the expression of genes such as Nalp6, which is known to inhibit Leydig cell testosterone synthesis, and decreased the expression of genes such as Npc and Lhcgr that up-regulate testosterone production. Within Sertoli cells, DBP increased the expression of *testin* and decreased Gja1 expression, both of which are associated with gap junction signaling, suggesting altered communication between Sertoli cells and gonocytes.

The dynamic nature of DBP-mediated effects is further illustrated by time course experiments in which DBP reduces testosterone production within 1 h of treatment, before detectable decreases in gene expression associated with cholesterol transport and steroid synthesis (62). DBP rapidly increased the expression of several genes, including members of the immediate-early gene family, which may play a role in the early decrease in testosterone production.

Although the mechanisms by which phthalate esters such as DBP alter the expression of genes required for normal male reproductive tract development have not been fully characterized, DBP serves as an example of an endocrine disruptor that elicits multiple effects on the male fetal reproductive tract, including antiandrogenic effects, without physically associating with the AR or altering AR-mediated signaling. Interestingly, recent studies have shown that DBP can activate the constitutive androstane receptor (CAR), the pregnane X receptor (PXR) (63), and the peroxisome proliferator-activated receptor subtypes (PPARα, -β, and -γ) (64), all of which are nuclear receptors (NRs) concentrated in the liver that regulate several metabolic enzymes, including those involved in steroid metabolism. These findings suggest that phthalate-induced effects on the male reproductive tract may be mediated by one or more of these NRs; however, additional research, perhaps using the CAR, PXR, and PPAR knockout mice, is needed to address this possibility.

**MAA**

MAA is the major metabolite of ethylene glycol monomethyl ether (EGME), an industrial solvent commonly used in varnishes, paints, dyes, and fuel additives (65). Exposure to EGME and MAA results in toxic reproductive effects in both animals and humans (66–71). Occupational exposure to both EGME and MAA has been associated with subfertility, spontaneous abortion, and reduced sperm counts (70, 72–74). The toxic effects of MAA have prompted investigations into the cellular and molecular actions of MAA that have uncovered unique actions for an EDC.

**In vitro actions**

*In vitro* studies show that MAA exerts its effects by potentiating the ligand-induced transcriptional activity of ERα,
ERβ, thyroid hormone receptor (TRβ), progesterone receptor (PR), and AR, indicating that MAA may influence the activity of a shared component of NR signaling (75, 76). However, MAA has negligible effects on NR-mediated transcriptional activity in the absence of hormone, indicating it is not functioning simply as a NR agonist. This was confirmed by receptor-binding assays, which showed that MAA does not compete with estradiol for binding to the ER (76). The potentiation effects of MAA are also not a result of the derepression of gene transcription, because microarray data show that MAA alone altered the expression of only three transcripts after a 24-h exposure. In the same experiment, addition of the PR agonist R5020 potentiated the PR-mediated expression of 16 genes and resulted in the induction of four new genes, indicating that MAA can alter NR target gene specificity.

In vivo actions

Additional experiments were performed to determine whether MAA had similar effects on NR function and gene expression in vivo. Immature CD-1 female mice were treated with the synthetic progestin R5020 in the absence and presence of MAA, and uterine levels of calcitonin mRNA expression were determined. Results showed that neither R5020 nor MAA alone had any effect on calcitonin expression, but a combination of the two produced an approximate 4-fold increase in expression, indicating MAA was capable of potentiating ligand-dependent NR-mediated transcriptional activation in vivo.

Mechanisms of action

The observation that MAA increases the transcriptional activity of multiple NRs suggests it may target a common component of NR action. One such potential target is the MAPK signaling pathway, which upon activation has been shown to potentiate the agonist-induced activity of both ERα and PR (54, 77). MAA increased the levels of activated Ras and ERK1/2 in HeLa cells, and a MAPK kinase inhibitor reduced the MAA-induced potentiation of agonist-bound PR by 60% in a reporter gene assay, indicating MAA is potentiating NR signaling through a MAPK-dependent signaling pathway (76). In addition to activating MAPK, MAA may regulate NR activity by modifying chromatin structure because MAA inhibits histone deacetylase activity in vitro and in vivo, resulting in increased levels of acetylated histone H4 (76).

The results of these studies reveal a novel mechanism of action for an EDC, in which MAA potentiates the ligand-dependent transcriptional activity of multiple NRs by targeting a common pathway(s) in NR-mediated signaling. This suggests that MAA exposure may potentiate the effects of weak nuclear receptor agonists found in the environment, producing a response indicative of a full agonist. Considering this possibility and the potential health impact associated with it, additional research evaluating the effects of MAA on NR activation by weak agonists, including environmental compounds, is needed.

Conclusions

DES, Gen, DBP, and MAA are four compounds described herein that illustrate the diverse biological effects and mechanisms of action used by EDCs to modulate endocrine system function. Studies on these compounds show that EDCs can act via receptor-mediated and/or non-receptor-mediated mechanisms to influence endocrine system function. The observation that EDCs can modulate the endocrine system in a receptor-independent manner has required investigators to reassess the criteria for classifying a compound as an EDC. The varied and sometimes complex mechanisms of action of EDCs, coupled with the physical and chemical diversity among members of the EDC family, suggest there may be numerous additional mechanisms used by EDCs that have yet to be uncovered. Future progress in identifying and characterizing EDCs will require an appreciation for their diverse mechanisms of action and will likely depend on the development of new screening methods and experimental models that account for this diversity.

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D.V.H. and K.S.K. have nothing to declare.

References

14. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O 1993 Alteration of reproductive function but not prenatal sexual development after
Henley and Korach • Mechanisms of Action of EDCs


55. Foster PM, Crayze DM, Foster JR, Gray TJ 1984 Testicular toxicity produced

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by ethylene glycol monomethyl and monoethyl ethers in the rat. Environ Health Perspect 57:207–217

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