ENVIRONMENTAL ENDOCRINE DISRUPTION: CAN THE ENDOCRINE DISRUPTION HYPOTHESIS BE VALIDATED IN THE INVERTEBRATE PHYLA?

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Summary

Anthropogenic chemicals, which can disrupt the endocrine systems of wildlife species, are currently a major cause for concern. In recent years, many instances have been recorded in which exposure to chemical contaminants has been linked to alterations in the sexual development and reproductive success of wildlife populations. These include changes in the sex of fish, reproductive failure in birds, and abnormal reproductive development in polar bears and alligators in polluted environments. Much less is known about the effects of endocrine disruption in the invertebrate phyla, despite the fact that alterations in the sexual development of certain mollusk species by the antifouling agent tributyltin represents one of the clearest examples of endocrine disruption to have been documented.

In this article, a brief overview is given of the endocrine disrupter hypothesis. Examples of potential endocrine disrupting chemicals are given and their likely mechanism of action described. The extrapolation of the concept of endocrine disruption to hormone systems other than the vertebrate-type sex steroids is discussed and the endocrinology of

several major invertebrate groups is considered. Examples of endocrine disruption in marine invertebrates *in situ* are provided and laboratory data presented concerning the effects of endocrine disrupters on a limited selection of invertebrate species. Current regulatory guidelines and developments are outlined and the problem of how to apply the present practices of ecological risk assessment to endocrine disrupting chemicals so as to protect effectively the diversity of wildlife is discussed. Finally, the importance of considering the hazard posed by endocrine disrupting chemicals in conjunction with the likely nature and extent of exposure experienced by species occupying different habitats and trophic levels (for example, water versus food, herbivores, carnivores, deposit feeders) is considered.

1. Introduction

In recent years, the international scientific community has increasingly addressed the question of whether anthropogenic environmental contaminants present a significant threat to human and ecosystem health. Governments, regulatory agencies, and scientists have responded to research suggesting that contaminant-induced alterations to chemical signaling pathways between cells may be a widespread phenomenon. The endocrine system integrates and directs many of the processes of growth, reproduction, and immunity through complex regulatory pathways. Many of the processes of endocrine signaling are ubiquitous across the animal and plant phyla, representing potentially vulnerable targets for the action of diverse chemicals. It is perhaps unsurprising that the concept of endocrine disruption has become such a widely studied and controversial topic for discussion. Books, reviews, discussion documents, and websites have proliferated at a near-exponential rate in the last few years, illustrating the extent to which this topic has gripped the popular imagination. Millions of pounds have been assigned or are targeted for future spending under the regulatory jurisdictions of the United States, Europe, Japan, Canada, and other countries to screen thousands of chemicals for their potential endocrine disrupting ability, following recommendations such as those of the US Environmental Protection Agency's 1999 *Endocrine Disrupter Screening and Testing Advisory Committee Final Report.* Yet it is still debatable from a review of the scientific evidence whether convincing and consistent epidemiological data exist to suggest that increased widespread adverse health effects related to environmental contaminants suspected of modulating endocrine function have actually occurred in human or wildlife populations.

Much interest has been directed at species associated with the aquatic food chain. A wide variety of environmental pollutants, including petroleum derived and chlorinated hydrocarbons and heavy metals, are discharged into rivers and estuaries and together with domestic sewage can accumulate in estuaries and marine waters. Aquatic invertebrates are the key structural and functional components of marine and freshwater ecosystems. One of the few reliable examples of endocrine disruption for which the decline of a population can be linked to a chemical suspected of altering endocrine function is tributyltin (TBT)-induced imposex in gastropod mollusks. TBT, the active ingredient in anti-fouling paints, causes interference with testosterone metabolism, leading to alterations in reproductive morphology and hence the population success of exposed mollusk communities. Given the ecological significance of this example, proportionately little research effort has addressed the issue of endocrine disruption in

aquatic invertebrates or the mechanisms by which invertebrate endocrine systems are regulated.

In this article, a brief overview of the endocrine disruption hypothesis is given. The potential mechanisms of action and effects of different classes of chemicals considered to be capable of causing endocrine disruption are reviewed and some of the latest facts and figures on endocrine disruption and reproductive failure specifically relating to aquatic invertebrates are considered. The ecological significance of endocrine disruption in marine invertebrates is discussed and gaps in our knowledge concerning the availability and uptake of endocrine disrupters in aquatic ecosystems are considered. Laboratory testing methods for detecting and screening chemicals for potential endocrine disrupting activity are described and an evidence-based approach for environmental management action is proposed, taking account of the latest regulatory guidelines. Finally, some of the trends and future perspectives emerging from recent discussions and publications are outlined.

2. Endocrine Disruption: A Threat to Ecosystem Health?

It has been known for centuries that certain plant products have the potential to interfere with human reproductive processes and the historical use of these phytoestrogens and mycoestrogens as contraceptives and aborticides is common to many cultures. A paper in the journal *Nature* in 1938 identified estrogenic activity in certain synthetic compounds, highlighting to the scientific community the potential of exogenous substances to interfere with endocrine systems. The immense amount of scientific, public, and commercial interest in recent years in the topic of anthropogenic reproductive disturbance is commonly attributed to the work of Rachel Carson in 1962, and more recently to Theo Colborn and colleagues in 1996 who described reproductive disorders and gave examples of the apparently global occurrence of endocrine disruption in humans and wildlife. These popular interest books have engendered strong reactions, both enthusiastic and skeptical, largely due to their tendency towards oversimplification of the science and a certain lack of scientific objectivity. The substantial literature that has accumulated on the topic of endocrine disruption includes documented changes in human sperm counts, genital tract malformations, infertility, increased frequencies of mammary, prostate, and testicular tumors, and retarded intellectual development in children, all of which have been attributed to environmental factors.

A review of the scientific data and criteria used to support this hypothetical association between human health effects and exposure to anthropogenic chemicals presented at a US Environment Protection Agency sponsored workshop in 1996 concluded that the relationship between endocrine effects in humans and exposures to environmental chemicals was far from clear. Despite the experimental demonstration of similar abnormalities of reproductive function in laboratory animals exposed to levels of environmental estrogens similar to amounts to which humans are commonly exposed, there remains to date no concrete evidence of a causal relationship between environmental contaminants and human cancers in hormonally sensitive tissues. The debate over the magnitude of endocrine disruption as a global concern to human populations is far from complete.

Several disorders have been described in wildlife populations and attributed to endocrine disruption. Vitellogenin is a female-specific egg yolk protein not normally produced in males. Induction of vitellogenin in male fish has been described in fish exposed to estrogenic compounds and in fish exposed to water polluted by sewage treatment works. Female fish exhibiting male characteristics have also been described following exposure to environmental waste. Studies in the United Kingdom have subsequently established that sexual development was affected in indigenous fish populations living in rivers receiving treated sewage effluent, with a high prevalence of intersex, in which alterations in the development of the gonadal duct and/or germ cells occur. Significant correlation between the severity of the intersex condition in roach and the level of exposure to treated sewage effluent provided strong evidence that the effluent or the chemicals it contained caused the development of intersex. Intersex in wild fish has also recently been shown in polluted waters in continental Europe, the United States, and Japan. Studies have shown that wild intersex roach have altered sex steroid hormone profiles, altered spawning time, and reduced sperm production, and are compromised in their reproductive capacity, producing sperm with poorer mobility and with a lower fertilization success. This in turn shows that intersex in wild fish has potential consequences at the population level.

Alligator populations from Lake Apopka, Florida, an area heavily contaminated with the pesticide DDT (dichlorodiphenyltrichloroethane) and other agricultural chemicals, experienced a decline in population which was mirrored by decreases in the testosterone levels and genital size of the males of affected populations. Similar reproductive abnormalities have been seen in polar bears. Reproductive failures have been found in seals and correlated with increased levels of PCBs in their body tissues. In turtles, gender development is a temperature-dependent process, but turtle eggs exposed to PCBs during the developmental process adopted a female outcome irrespective of temperature.

A review of the scientific evidence suggests that high doses of some environmental contaminants have produced toxic effects in certain wildlife species, with an involvement of endocrine mechanisms as the toxicological mode of action. However, the ecological relevance of these effects at the population level has yet to be proven unequivocally.

3. Endocrine Disrupting Chemicals

The term endocrine disrupter has been used interchangeably with other similar terms including endocrine modulating substance, endocrine active chemical, hormonally active agent, environmental hormone, xenoestrogen, and gender-bender (the latter a particular favorite of the tabloid press). For the purposes of this article, the term endocrine disrupter will be used. Controversy surrounding an exact definition of this term is illustrative of the debate surrounding the whole endocrine disruption issue. Neither the US Environmental Protection Agency's Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) nor the Organisation for Economic Cooperation and Development (OECD) could initially agree on a suitable definition and instead relied on a "description." Various and diverse interpretations of what constitutes

an endocrine disrupter have been given in the literature. A consensus description of endocrine disruption (used by most relevant agencies, for instance the UK Medical Research Council and the Danish Environmental Protection Agency) is the disturbance of hormone function in humans and other animals caused by exposure to low levels of anthropogenic chemicals. Reproductive hormone-receptor systems appear to be especially vulnerable. The endocrine and reproductive effects of pollutants are believed to be due to their ability to:

- mimic the effects of hormones
- antagonize the effects of hormones
- alter the pattern of synthesis and metabolism of hormones
- modify hormone receptor levels

At present the endocrine disrupters receiving the most attention are those that, despite having widely diverse chemical structures, mimic the actions of endogenous estrogen. These so-called xenoestrogens, estrogenic xenobiotics, or exoestrogens may cause their effects through interaction with the estrogen receptor or through other mechanisms. An extensive list has been assembled of chemicals thought to be capable of disrupting the reproductive endocrine systems of animals. Some examples of chemicals suspected of causing endocrine disruption, grouped according to their likely mode of action, are shown in Table 1.

Proposed mechanism of action	**Examples**
Estrogen receptor mediated	Methoxyclor, bisphenolic compounds, o,p'-DDT
Anti-estrogenic	Dioxin, endosulphan
Anti-androgenic	Vinclozolin, DDE, Kraft mill effluent
Modulation of circulating steroid hormone levels	Fenarimol and other fungicides, endosulphan, dioxin, Aroclor 1254, TBT
CNS mediated effects on reproduction	Dithiocarbamate, methanol, pesticides
Anti-thyroid endocrine disruption	Pthalic acid esters, herbicides e.g. nitrofen, PCBs
Adrenal endocrine disruption	Aniline dyes, ketoconazole fungicides, PCBs
Other diverse mechanisms	Cadmium, dibutyl pthalate, benzidine-based dyes, vinylcyclohexene

(Abbreviations: o,p'-DDT = 1,1-dichlorodiphenyltrichloroethane, DDE = 1,1-dichloro-2,2-bis (p-chlorophenyl)ethylene, PCB = polychlorinated biphenyl, TBT = tributyltin.)

Table 1. Some examples of chemicals suspected of causing endocrine disruption grouped according to their proposed mechanism of action

The Agency for Toxic Substances and Disease Registry (ASTDR/EPA) priority list of the top 20 hazardous substances of most concern from a global perspective features a number of chemicals also suspected of causing endocrine disruption and these are shown in Table 2.

1. Arsenic
2. Lead
3. Mercury
4. Vinyl chloride
5. Benzene
6. Polychlorinated biphenyls (PCBs)
7. Cadmium
8. Benzo(a)pyrene
9. Polycyclic aromatic hydrocarbons
10. Benzo(b)fluoranthene
11. Chloroform
12. DDT, P'P'
13. Aroclor 1260
14. Aroclor 1254
15. Trichloroethylene
16. Chromium (+6)
17. Dibenz[a,h]anthracene
18. Dieldrin
19. Hexachlorobutadiene
20. DDE,P P'

Criteria used in the evaluation process can be accessed at <www.atsdr.cdc.gov/cxcx3.html>

Table 2. ASTDR/EPA priority list of top 20 hazardous substances (2001)

Many of these chemicals occur frequently in estuarine marine waters and may bioaccumulate at chronically polluted sites. For instance, the bioaccumulation concentration factor (BCF) for DDT in aquatic animals ranges from 25–100 x 10^3 whilst that of nonylphenol, a degradation product of alkylphenol polyethoxylates, a class of nonionic surfactant found in high levels in sewage effluents, is around 300. The chlorinated hydrocarbons, especially DDT and its metabolites and the polychlorinated biphenyls (PCBs), are two of the most intensively studied of these classes of chemicals in terms of their potential endocrine disrupting activity as well as for their additional lethal, carcinogenic, and harmful effects. Effective legislation in most developed countries has successfully reduced the levels of these compounds reaching the environment, and consequently the body burdens of PCBs and DDT encountered in higher trophic level species have declined from the mg kg^{-1} range previously reported in organisms inhabiting contaminated water bodies. Likewise, the decline in TBT-exposed mollusk populations has been partially reversed in many countries through regulatory action, illustrating the resilience of communities and ecosystems to perturbing influences.

While the ban on the production and usage of major organochlorine contaminant groups has resulted in a decline in environmental levels, environmental monitoring programs have highlighted increasing levels of other compounds of potentially harmful impact, for instance the polybrominated diphenyl ethers (PBDEs). PBDEs are used as additive flame-retardants and are present in relatively high concentrations in electronic equipment such as computers, in building materials, plastics, and textiles. PBDEs can migrate out of the product and cause diffuse contamination of the environment, showing similar behavior and toxicity profiles to PCBs and DDT. Of particular concern is their putative role in the disruption of thyroid function in vertebrate model systems, although their effects in invertebrates are largely undetermined. The total worldwide consumption of PBDEs doubled to over 4,000 tonnes per year in the eight years up to 1992 and a corresponding increase in environmental levels has been reported. For example, PBDEs have been located in sediments downstream of potential sources in rivers in the United Kingdom. Increasing concentrations have been reported in the tissues of fish, fish-eating birds, and marine mammals, illustrating the propensity of

these contaminants towards atmospheric transport and bioaccumulation through the food chain.

Domestic sewage effluents and lake waters in heavily populated areas can contain ecologically significant concentrations of natural and synthetic steroidal estrogens and their metabolites including 17 β estradiol and 17 α ethinylestradiol. The total quantity of natural estrogen excreted in the urine of a woman is typically around 60 μg per day rising to around 300 μg per day during pregnancy. The contraceptive pill contains about 30–40 μg of the synthetic estrogen ethinylestradiol. The latter compound has an estrogenic potency far greater than estradiol itself. Intensive farming practices include the use of synthetic compounds such as the anabolic agent zearanol, which also exhibits estrogenic properties. In many cases, the determination of routes of uptake, and patterns of bio-activation, biotransformation, and excretion of these compounds in diverse species has yet to be carried out. For instance, the metabolic hydroxylation of PCBs, which occurs in certain species, produces a range of hydroxybiphenyl metabolites of greater estrogenicity than the original PCB. There is still considerable debate as to how many of the compounds mentioned function in mixtures, especially in relation to whether they act synergistically, antagonistically, or additively. Recent studies suggest that the action of mixtures is at least additive.

4. Xenoestrogens: Mechanism of Action

As highlighted above, there are many potential ways in which disruption of endocrine function may occur. Reproductive hormone receptor systems appear to be especially vulnerable, and for this reason the cellular mechanisms of action of estrogenic compounds with the estrogen receptor are considered as an illustrative example. Endogenous estrogens are steroid hormones synthesized in gonadal tissue, which act on the cells and tissue of the female genital tract and other tissues to induce proliferation, and to induce estrous (mating) behavior in mammals by acting on the central nervous system. The cellular mechanism of action of estrogen in target cells is through interaction with an estrogen receptor protein located in the nucleus. Binding of the hormone induces a conformational change in the receptor that facilitates high affinity binding of the activated receptor to specific DNA sequences called estrogen response elements. These regulatory regions are associated with specific genes, altering their expression in a manner that can be modified by coactivators, repressors, and modulators (Figure 1).

Unlike many protein hormones, which may vary in amino acid sequence between species, estradiol is identical in vertebrate species and the estradiol-binding domain of the α estrogen receptor shows a high level of conservation across hundreds of millions of years of evolution. Thus a chemical capable of binding the estrogen receptor in one species can be predicted to bind in any other species in which that form of the receptor occurs, although the outcome will depend on species and tissue specific differences in the process of transcription. Occupation of the estrogen receptor by exogenous chemicals may therefore interfere with reproductive cycles and behavior or, during critical periods of early development, may lead to irreversible alterations in cell differentiation and organogenesis, altering the normal developmental program.

A diverse range of compounds are able to bind to the estrogen receptor making prediction of endocrine disrupting ability based on the quantitative structure activity relationship (QSAR) approach difficult. A phenolic hydroxyl group appears to be essential and the estrogenic properties of many chemicals are enhanced following metabolic hydroxylation. Yet while most xenoestrogens showing high receptor affinity possess a para-substituted phenolic ring, some have more than one (methoxychlor metabolites and diphenolic isoflavinoids) and others have none (chlordecone and o,p'-DDT) (Figure 2).

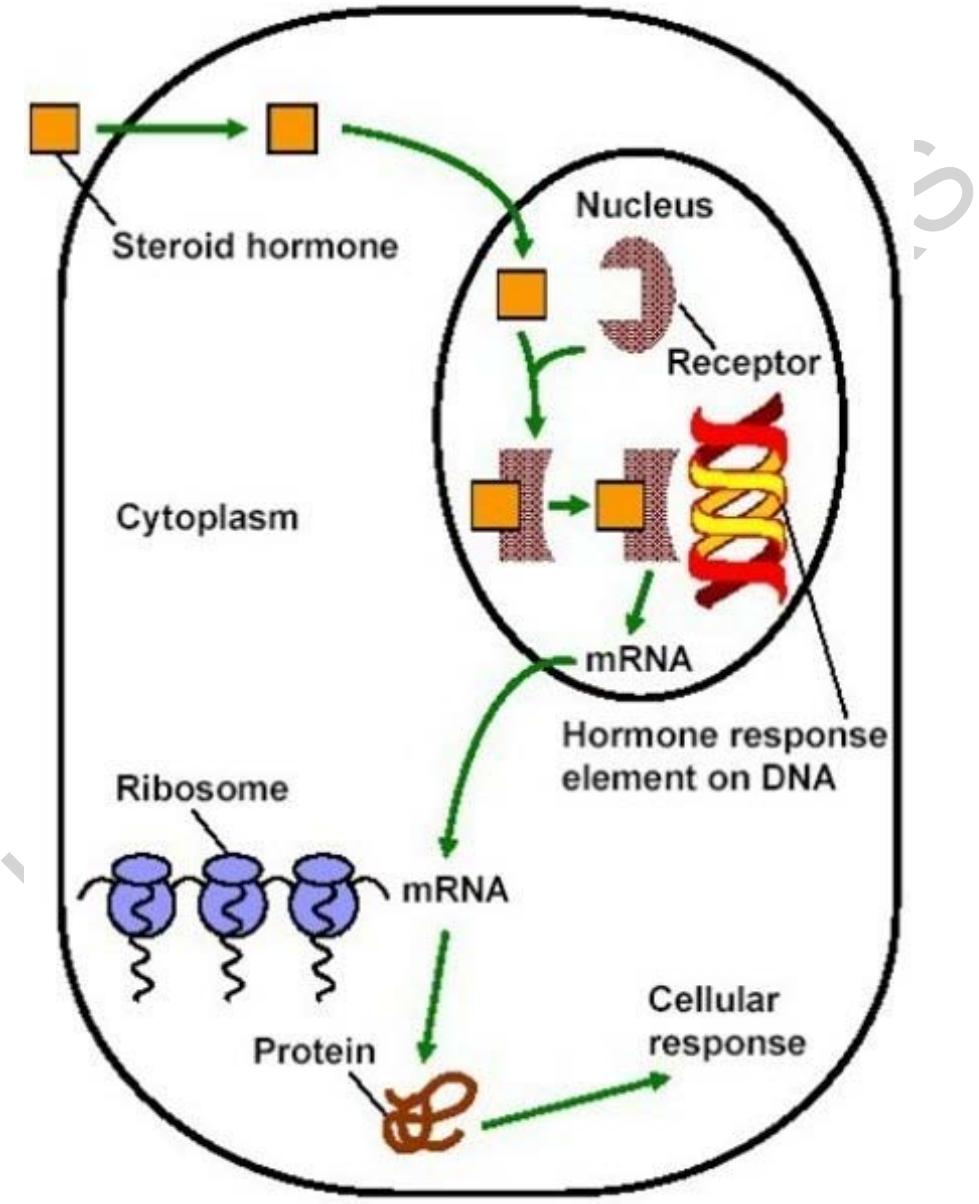

Figure 1. Cellular mechanism of action of estrogen

The estrogen receptor is a member of the steroid receptor superfamily comprised of receptors for steroids, thyroid hormones, vitamin D3, retinoic acid, and several so-called orphan receptors for which the ligand has yet to be identified. There is evidence for the

existence of several different steroid receptor subtypes within a single species, which may be differentially distributed and differentially activated. Cross-reactivity among various ligands and their corresponding receptors further complicates the interpretation of the ligand-receptor relationship.

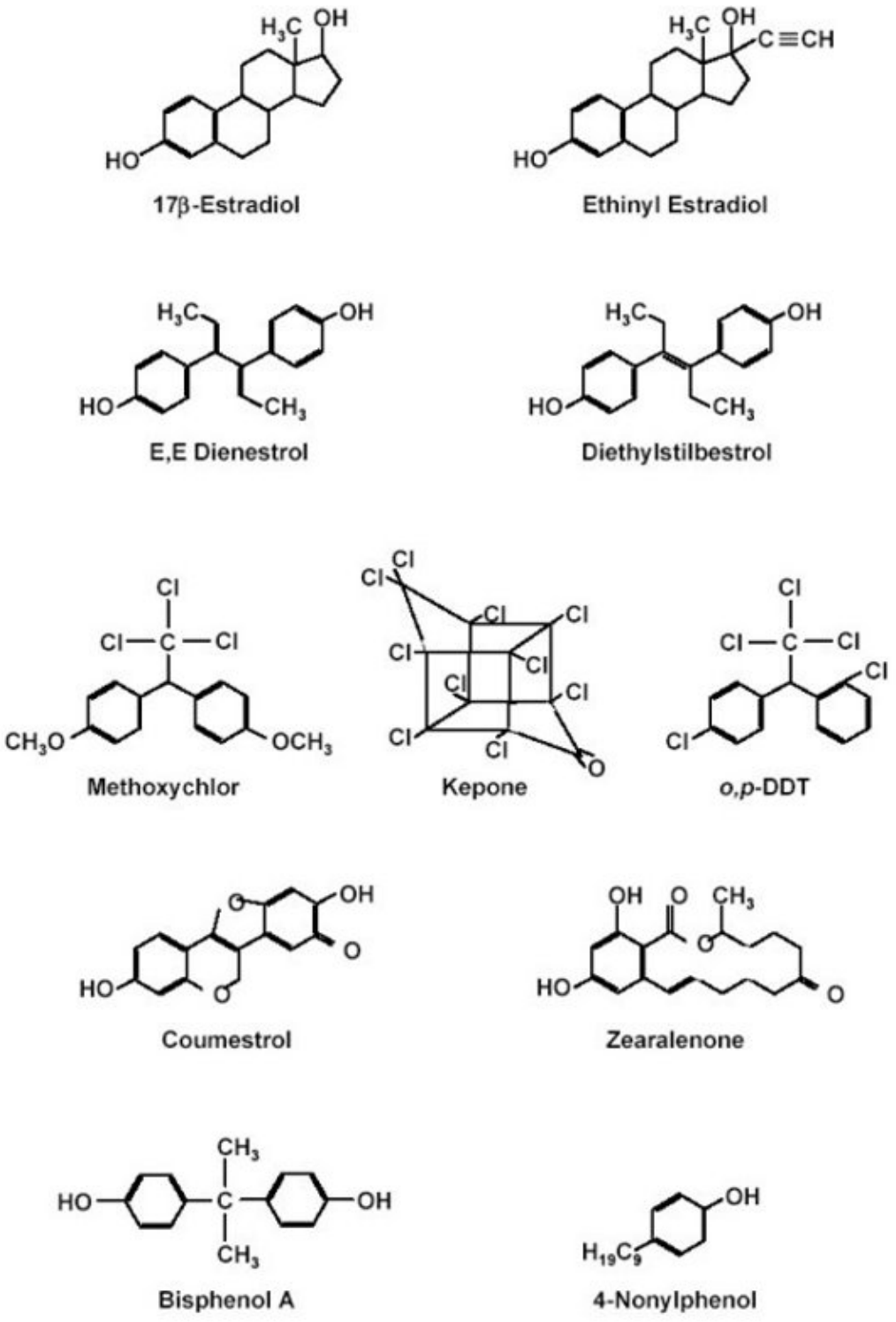

Figure 2. Structures of some chemicals exhibiting estrogenic activity

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