Functional Toxicology: A New Approach to Detect Biologically Active Xenobiotics

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The pervasiveness of chemicals in the environment with estrogenic activity and other biological functions recommends the development of new approaches to monitor and study them. Chemicals can be screened for activity *in vitro* using a panel of human or animal cells that have been transfected with a specific receptor and reporter gene; for example, the estrogen receptor. By using a variety of different receptors, the screening of xenobiotics for biological functions can be broad. Chemicals could then be classified by their function *in vitro* which, in some cases, may be a useful guide for toxicological studies. *Key words:* estrogens, functional toxicology, hormone receptors, xenobiotics. *Environ Health Perspect* 101:386–387 (1993)

The preceding papers by Davis et al. and Colborn et al. describe chemicals in the environment that may affect the immune, nervous, and endocrine system in ways that are potentially deleterious to human health or the health of a wide range of animals. Many of the chemicals described exhibit biological activities associated with the female sex hormone estrogen. The issue of biologically active environmental chemicals was raised at a symposium at NIEHS in 1979, "Estrogens in the Environment" (*1*), in which concern for the widespread distribution of advertent hormones in the environment like diethylstilbestrol (DES), previously used in the cattle industry, and inadvertent hormones in the environment, such as DDT and other xenobiotic estrogens, were summarized and evaluated. At that meeting, the possibilities for foreign chemicals to exert specific biological effects were clearly established. In fact, as shown in Figure 1, the theoretical basis for environmental chemicals to exert hormone-like effects is relatively straightforward. In the simplest model, chemicals can mimic a hormone by binding to its receptor and eliciting a spectrum of biological effects. Conversely, a foreign chemical that does not elicit these effects could bind a hormone receptor as an inactive compound and thus block the response to the natural hormone. In either case, the result would be an alteration in the function of the hormone system. In actuality, the processes involved are much more complex.

Many chemicals with different structures were shown to exhibit weak estrogen-like functions (Fig. 2). In addition to these diverse structures reported to be estrogenic, other chemicals have been discovered. A study from our laboratory demonstrated the estrogenic potential of some hydroxylated forms of polychlorinated biphenyls (PCBs) (*2*). More recently, the estrogenic substances *p*-nonyl-phenol (*3*) and bisphenol-A (*4*) have been shown to be released from plastic under a variety of conditions.

However, at the time of the 1979 meeting there were few, if any, documented reports of human health effects from xenoestrogens. It wasn't until the next NIEHS meeting, "Estrogens in the Environment" in 1985 (*5*) that a body of data was presented which addressed the effect of environmental estrogens on puberty in young children. The association between environmental factors and precocious puberty remains unestablished. However, concern was expressed about the ubiquitous nature of these materials, their discordant potency, and their potential impact for public and environmental health. A meeting held in 1992, organized by the World Wildlife Fund, explored the premise that these compounds may have a deleterious effect on sexual development in a variety of wildlife species (*6*). While these reports remain largely anecdotal at the present time, they again raised the possibility that these molecules may have biological effects.

For environmental estrogens, one may consider the work done with DES in experimental systems, where there are human studies for comparison, as a prototype. In both experimental animals and humans, prenatal exposure to the potent estrogen DES is associated with, among other changes, vaginal cancer, paraovarian cysts, structural abnormalities of the uterus and oviducts, and reproductive dysfunction in the female offspring (*7*).

As with female offspring, male mice exposed prenatally to DES express reproductive tract lesions as adults which include increased infertility, testicular abnormalities inconsistent with normal spermatogenesis, epididymal cysts, and undescended testes. Similar findings have been reported for humans similarly exposed to DES *in utero* (*7*).

Exposure to estrogen during sexual development is also associated with feminization of the male reproductive system in mice (*8*) as well as in numerous other mammalian, reptilian, and avian species; this, as yet, has not been reported in humans. Certainly, the lesions associated with developmental exposure to a potent estrogen provide indicators for potential effects associated with much weaker environmental estrogens.

Although the possibilities for human health effects of hormonally active foreign chemicals remain theoretical, additional recent reports add to the growing sense that some diseases or dysfunctions associated with estrogens may have an environmental component. For example, a recent report (*9*) suggests an association between organochlorines and breast cancer, and, in a commentary on the analysis that reported a significant decrease in human sperm count over the last 50 years (*10*), the author speculates that this apparent drop in sperm may

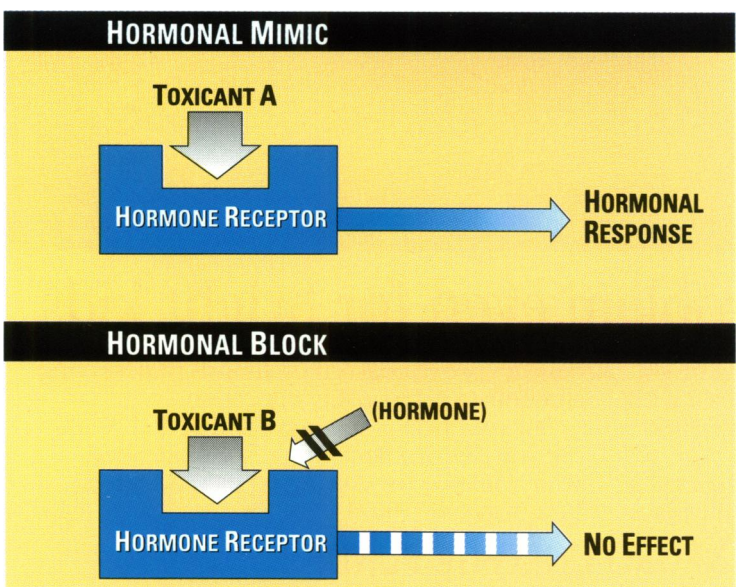

Figure 1. Exogenous chemicals may act at hormone action sites.

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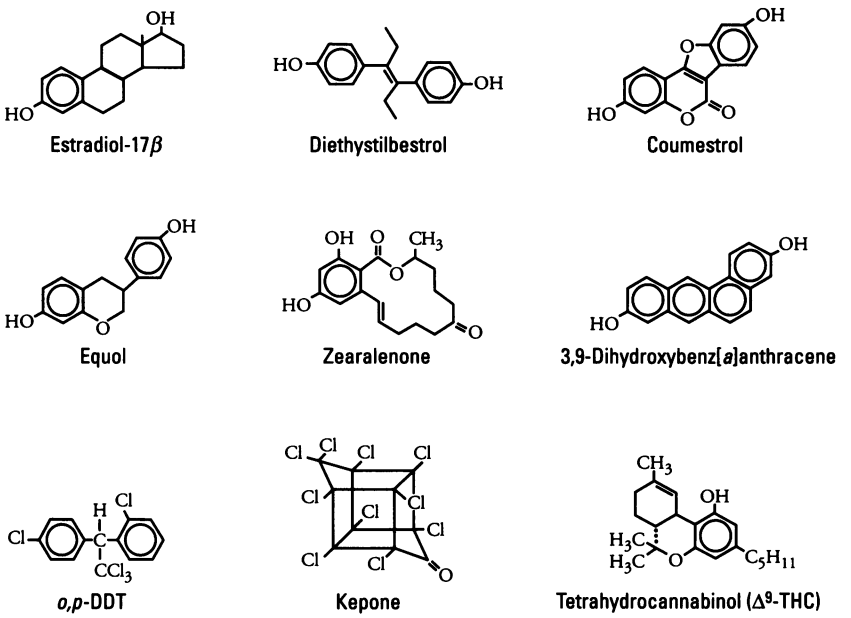

Figure 2. Chemicals reported to be estrogenic: steroidal "natural" ovarian estrogens (estradiol); synthetic potent estrogen (diethylstilbestrol); plant or phytoestrogens (coumestrol); estrogenic metabolites of plant substances (equol); mycotoxin estrogens (zearalenone); polycyclic aromatic hydrocarbon "prohormone" (dimethylbenzanthracene); chlorinated hydrocarbon "prohormone" (*o,p*-DDT); fully chlorinated "bioaccumulative estrogen" (kepone); "indirect" estrogen (tetrahydrocannabinol). See McLachlan et al. (*13*) for further details.

be due to developmental exposure to estrogenic xenobiotics (*11*).

These issues of biologically active xenobiotics have raised the need for a "new toxicology" using available methodologies. A great number of inter- and intracellular signals for cell differentiation, proliferation, and function involve the interaction between small molecules and their receptors. A class of receptors that recognize most steroid hormones, thyroid hormones, retinoids, and some vitamins are nuclear transcription factors involved in gene regulation (*12*). This class and related families of genes also recognize some foreign chemicals, such as dioxin. Thus, some xenobiotics may exert their effects, in part, through their interaction with a nuclear receptor and activation of the genes it regulates. In the case of chemicals which interact with the estrogen receptor, it is clear that their activity is defined more by their function (estrogenicity) than by their structure. Thus, an understanding of receptor-mediated action of chemicals may help to define a function of potential toxicological importance.

To this end, we can suggest a research strategy referred to as "functional toxicology," in which chemicals are defined more by their function than by their chemistry. As part of this strategy, cells, including human cells, should be transfected with molecular constructs containing a specific receptor and the reporter gene for that receptor. One then can assay in a relatively quick and straightforward way not only the binding of a chemical ligand to the receptor, but the receptor occupancy of its response element and gene activation. Thus, if a panel of receptor-containing cells were set up, one could screen chemicals of unknown biology or toxicology and determine their function, at least in regard to receptor-mediated gene activation. A related functional *in vitro* screen for estrogens has been suggested by Soto et al. (*6*).

Screening for functionality could be made fairly broad by having the panels include the estrogen receptor, progesterone receptor, androgen receptor, glucocorticoid receptor, retinoid receptor, thyroid hormone receptor, peroxisome proliferator receptor, dioxin receptor, excitatory amino acid receptors, and so on. At the end of such an analysis, one could determine if a chemical of unknown biology and toxicology exhibited a known, measurable biological function. For a number of chemicals, one could assign, in addition to melting point, molecular weight, and solubility, a functional description. This information could have public health significance because by understanding the functional basis of some toxicological responses, careful biological generalizations could be attempted. That is, a chemical that is DES-like will have more predictable, understandable toxicities associated with it, depending upon its dose, duration, and time of action based on decades of experience with the prototype compound and its paradigmic effects. Likewise, an unknown chemical functioning like retinoic acid, would have a different, yet, to some extent, predictable, set of toxicities associated with it. Chemicals that were dioxinlike would have another set of toxicities associated with them; excitotoxins that interact with the NMDA, or excitatory amino acid receptor, would have another set of expected outcomes. Although this strategy would in no way provide a complete toxicological profile of a chemical or replace full animal testing or epidemiology and public health strategies, it would generate essential, useful information for an important set of toxicological problems in a relatively short time and at relatively low cost. For example, using this strategy one could determine what chemicals in the environment are DES-like and what their relative potency would be, at least, in an *in vitro* gene-activation assay. Then one could develop further a plan to target either additional tests or public health approaches for these chemicals based on their functional toxicology.

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