



## Guest Editorial

### Interactive Hormonal Activity of Chemical Mixtures

Public and legislative concern that chemicals in our environment may be affecting human health by mimicking natural hormones and disrupting normal endocrine function remains focused on estrogenic activity. This has fueled the development of a number of *in vitro* assays to screen chemicals for estrogenic activity including the activity of chemical mixtures. There is controversy over the potential of such chemicals in combination to have synergistic properties (1,2). Many/most of these assays reflect the importance of high-affinity receptor proteins located within target cells in mediating the biological action (3). Endocrine system disruption implies interference with a complex multicomponent process. Existing studies may be overly focused on estrogenic activity and reproductive and developmental endpoints, in addition to having other limitations.

In the first place, proper selection of the chemicals to study is likely to be crucial for demonstrating any type of interactive effects. This is based on the fact that natural hormones and their agonists contain multiple molecular recognition domains (such as the A and D-ring domains in estradiol), which are distinct in the overall expression of activity (4). Hormone mimics (full agonists) must put into play a total interaction energy comparable to that of the natural hormone. The multifunctionality of hormones in molecular recognition processes may be important in dimerization or domain clustering processes involving either of two fundamental mechanisms by which information is relayed from one macromolecule to another in the cell; one involves allostery and the other proximity (5). These molecular properties are not limited to halogenated hydrocarbons, even though there has been preoccupation with this broad class of chemicals in endocrine disruptor projects funded by federal agencies (6). At a minimum, the chemicals selected for mixture study should represent structures containing each of the important recognition domains as well as combinations thereof. In addition, the potentially more important issue of antagonism (7) of natural endogenous hormones has been largely neglected. Antagonism implies a lower degree of molecular recognition and specificity because an antagonist only needs to block access to a receptor (4). The environmental significance of such probe mixtures in a real world contamination sense is another issue. However, identifying the important molecular recognition domains involved is important in delineating the molecular mechanism of action and could help reveal unrecognized chemicals/classes and combinations thereof that might be of environmental importance.

Perhaps more importantly, *in vitro* systems are incomplete systems. Although useful information can be obtained from these studies, special care has to be exercised in relating it to the more complex issue of endocrine system disruption. This is the case not only for *in vitro* systems involving measurement of a single type of hormonal activity but is especially a problem (even in *in vivo* systems) in the

*“There clearly is a need to expand our thinking about ... how best to address possible human health risk associated with exposure to chemical mixtures with potential for hormone mimetic chemical interactions.”*

context of measuring biological responses regulated by multihormonal interactions for which there are documented examples (8). The recent research article in *EHP* by Benjamin Danzo (9) is getting more to the heart of the issue of involvement of multihormonal systems. His data indicate that xenobiotics, even within a single chemical class, can affect multiple signaling pathways. The ability of any one ligand or ligand class of closely related congeners to compete effectively for more than one receptor implies a degree of conformational adaptability and flexibility exceeding that of the natural hormones. It is this flexibility derived from energetically accessible conformations that appears to characterize the ability of many chemicals of environmental concern to act as hormone mimics. Chemicals with favorable low energy conformational flexibility and complementary receptor protein flexibility may bind quite effectively to initiate a biological response (10), although not all bound conformers are necessarily equivalent in their ability to elicit a hormonal response. However, conformational restriction can favor binding for compounds having a close structural relationship (11). Interactions of the same chemicals or chemical class members with the same receptor systems and different chemicals or chemical classes with different receptor systems are both important research issues here. In this context, there are cross-reactivity binding data with several classes of steroid receptors to suggest that the estrogen receptor is the least tolerant of the steroid receptor family of proteins (12). In addition, a single chemical occupying one receptor can indirectly affect others by, for example, up/down regulation of receptor numbers and affinity. Dioxin provides one of the best known examples of one chemical affecting multiple signaling pathways (13).

In addition to these considerations, other factors can come into play such as favorable binding kinetics and the need to study the cellular and tissue dosimetry and kinetics of hormonelike chemicals in target organs relative to concentrations of natural hormones at critical points in time, such as in development and reproduction. There clearly is a need to expand our thinking about this problem area and how best to address possible human health risk associated with exposure to chemical mixtures with potential for hormone mimetic chemical interactions. Although many/most of these chemicals may function as imperfect hormones with relatively low potencies, we have not begun to understand what the potential adverse effects are of being exposed continuously to complex mixtures of chemicals with varying abilities to affect multiple signaling pathways both singly and interactively.

**James D. McKinney**

U.S. Environmental Protection Agency  
Research Triangle Park, North Carolina

*Note: These views are those of the author and not necessarily those of the U.S. EPA.*

## REFERENCES

- Gaido KW, McDonnell DP, Korach KS, Safe SH. Estrogenic activity of chemical mixtures: Is there synergism? *CIIT Activities* 17(2):1-7 (1997).
- Wiese TE, Lambright C, Kelce W. Lack of synergistic estrogen effects of dieldrin and endosulfan mixtures on MCF-7 and MVLN cells. *Toxicologist* 36:294 (1997).
- Ing NH, O'Malley BW. The steroid hormone superfamily: molecular mechanisms of action. In: *Molecular Endocrinology: Basic Concepts and Clinical Correlations* (Weintraub BD, ed). New York:Raven Press, 1995;195-215.
- McKinney JD, Waller CL. Molecular determinants of hormone mimicry. Halogenated aromatic hydrocarbon environmental agents. *J Toxicol Environ Health* (in press).
- Austin DJ, Crabtree GR, Schreiber SL. Proximity versus allostery: the role of protein dimerization in biology. *Chem Biol* 1:131-136 (1994).
- Forum: Priorities for endocrine disruptor research. *Environ Health Perspect* 105:372 (1997).
- Kelce WR, Wilson EM. Environmental antiandrogens: developmental effects, molecular mechanisms, and clinical implications. *J Mol Med* 75:195-207 (1997).
- Nyborg JK, Nguyen AP, Spindler SR. Relationship between thyroid and glucocorticoid hormone receptor occupancy, growth hormone gene transcription and mRNA accumulation. *J Biol Chem* 259(20):12377-12381 (1984).
- Danzo BJ. Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. *Environ Health Perspect* 105:294-301 (1997).
- Vajda S, Weng Z, Rosenfeld R, Delisi C. Effect of conformational flexibility and solvation on receptor-ligand binding free energies. *Biochemistry* 33:13977-13988 (1994).
- Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol Pharmacol* 33:120-126 (1988).
- Deletre J, Mornon JP, Lepicard G, Ojasoo T, Raynaud JP. Steroid flexibility and receptor specificity. *J Steroid Biochem* 13:45-59 (1980).
- Birnbaum LS. The mechanism of dioxin toxicity: relationship to risk assessment. *Environ Health Perspect* 102(suppl 9):157-167 (1994).

# NIEHS Excellence in basic research at the National Institute of Environmental Health Sciences

NIEHS scientists and grantees perform basic studies of our susceptibility to environment-related disease: Demonstrating that a carcinogen in cigarette smoke (benzo(a)pyrene) alters part of a gene to cause lung cancer...showing the effects of fetal exposure to PCBs...developing a strain of mouse that lacks functional estrogen receptors and that helps evaluate how some pesticides and other estrogen-like compounds might affect development and reproduction...discovering the genes for breast, ovarian, and prostate cancers...finding women's optimal days of fertility...seeking to reverse the damage from lead exposure...finding alternatives to traditional animal tests...pinpointing the functions of specific genes by eliminating them from specially bred mouse lines...discovering how using ordinary yeast cells, to isolate and clone genes and other fragments of genetic material...studying the effects of urban air on lung function...

