Human Health and Chemical Mixtures: An Overview

David O. Carpenter, Kathleen F. Arcaro, Brian Bush, William D. Niemi, Shaokun Pang, and Dilip D. Vakharia

Department of Environmental Health and Toxicology, School of Public Health, University at Albany, Rensselaer, New York; Wadsworth Center, New York State Department of Health, Albany, New York

Unlike laboratory animals, people are rarely exposed to a single hazardous chemical. However, most of the information documenting adverse human health effects from environmental and occupational contaminants has come from studies focused on exposure to single chemicals, and there is little information available on how two or more contaminants affect humans. Most information on the effects of mixtures comes from animal systems and limited investigations of isolated human cells in culture, even though the study of mixtures in such systems has also been neglected. Two or more compounds may show additive, antagonistic, or synergistic interactions or may act on totally different systems and thus not interact. Furthermore, even a single chemical may have multiple effects and affect more than one organ system. Effects may vary with age, and metabolites may have totally different actions from the parent compound. This paper will review the variety of health effects in humans that may result from environmental contaminants and discuss how such contaminants may interact with each other. We will also present examples on how different contaminants interact from toxicologic studies of polychlorinated biphenyls performed as part of our Albany, New York, Superfund Basic Research Program project. — *Environ Health Perspect* 106(Suppl 6):1263–1270 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-6/1263-1270carpenter/abstract.html*

Key words: metals, PCBs, estrogen disruptors, thyroid, cancer, birth defects, persistent organics, neurobehavioral effects

Human exposure to environmental contaminants is omnipresent. It does not occur only in individuals who live next to hazardous waste sites or just the disadvantaged and poor who live in inner cities or third-world countries. Everyone carries a burden of lead in his or her bones, mercury in their hair, and dioxins and polychlorinated biphenyls (PCBs) in their body fat. Environmental contamination is a global issue, and contaminants in one country often are transported to others via air, water, foodstuffs, manufactured products, and travelers. Environmental contaminants may be natural substances such as metals or radioactive materials, or they may be manufactured products that are useful to humans but still have toxic effects. Contaminants may be manufactured or they may be unintentional by-products of human activity, as in the case of combustion or incineration products or the generation of chloroform as a result of chlorination of drinking water. Many contaminants are mixtures of related chemicals such as polycyclic aromatic hydrocarbons (PAHs), crude and refined petroleum products, and polychlorinated aromatics; in some of these cases even the individual components have not been examined for toxicity.

As documentation of the widespread degree of contamination, especially in developed countries, Figure 1 shows a chromatogram of urine extracted from wet diapers of two infant boys 1 year of age. The peaks in the chromatogram are identified single PCB congeners and the dichlorodiphenyltrichloroethane metabolite dichlorodiphenyldichloroethylene. One infant (upper trace) was breast fed, while the other (lower trace) was not. The analyses were conducted to determine whether the breast-fed infant had more PCBs and pesticides in his urine than the non-breast-fed infant, as breast milk reflects the composition of lipophilic substances present in a mother's body fat. What is important is that these chromatograms show that both infants at 1 year of age already had significant evidence of exposure to a large number of chemicals even though their dietary intake at this age was limited. These substances are lipophilic and are retained in body fat. What is excreted in urine is only a small reflection of total exposure and body burden. The question of importance for the health of these boys is not simply what chemical X does to their development and health, but rather what the impact is of all of these chemicals acting together.

Health Effects of Mixtures

Some of the major broad categories of human diseases that are suspected to result from exposure to environmental contaminants are cancer, birth defects, immune system defects, reduced intelligence quotient (IQ), behavioral abnormalities, decreased fertility, altered sex hormone balance, altered metabolism, and specific organ dysfunctions (*2*). Almost every organ system may be affected by one or more substances commonly found in our environment. The diseases listed are abstracted from many studies of both humans and animals, and in most cases these investigations were focused on a single contaminant. Some of these diseases, when expressed in a given individual, are difficult to ascribe with certainty to a particular exposure (*3*). This is true for cancer, birth defects, and many of the endocrine disruptor and nervous system actions. But others, such as the specific organ system dysfunctions seen with kidney disease following lead exposure (*4*), or the loss of particular neurons following methylmercury exposure (*5*), are clearly attributable to particular exposures. Many of the effects of contaminants on humans

This paper is based on a presentation at the Conference on Current Issues on Chemical Mixtures held 11–13 August 1997 in Fort Collins, Colorado. Manuscript received at *EHP* 17 February 1998; accepted 24 June 1998.

Supported by National Institute of Environmental Health Sciences Superfund Basic Research Program grant P42 ES04913.

Address correspondence to D.O. Carpenter, School of Public Health, One University Place, Rensselaer, NY 12144. Telephone: (518) 257-2025. Fax: (518) 525-2665. E-mail: carpent@cnsvax.albany.edu

Abbreviations used: Ah receptor, aryl hydrocarbon receptor; CYP, cytochrome P450; DES, diethylstilbestrol; E_2, 17β-estradiol; β-gal, β-galactosidase; hER, human estrogen receptor; EC_{50}, median effective concentration; HxCB, hexachlorobiphenyl; IC_{50}, concentration that inhibits 50%; IQ, intelligence quotient; LTP, long-term potentiation; PAHs, polycyclic aromatic hydrocarbons; PB, phenobarbital; PCB, polychlorinated biphenyl; PeCB, pentachlorobiphenyl; PTU, propylthiouracil; 2,3,7,8-TCDD, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin; TeCB, tetrachlorobiphenyl; TEFs, toxic equivalent factors; Th, T helper; TrCB, trichlorobiphenyl.

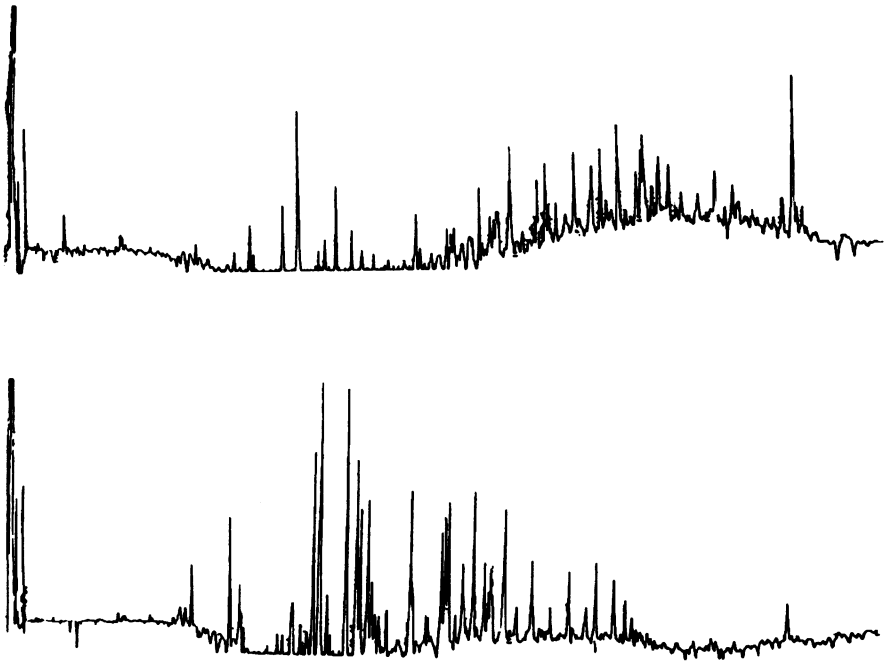

Figure 1. Gas spectrometry chromatograms of urine extracts from diapers from two infants at 1 year of age, showing presence of various single PCB congeners and pesticides. Analysis was made as described by Bush et al. (*1*). The infant in the upper trace was breast fed, while that in the lower trace was not. Each peak is an identified PCB congener or pesticide.

are subtle and difficult to quantify. This is particularly true for alterations that occur during development and are thus irreversible, as are many of the effects on the nervous system and organs that are hormonally regulated.

There are a number of factors that complicate the toxicologic evaluation of mixtures. Two or more compounds may have additive effects as a result of acting at the same site, altering the same process by different mechanisms, or as a result of one compound altering the metabolism of the second in such a way as to generate a toxic metabolite. They may also have antagonistic effects or may be synergistic in that the two together give much greater response than the sum of either alone. However, they also may have absolutely no interactions, with each substance acting independently. In this case the net effect of the lack of interaction is that a person experiences the sum of the different organ toxicities of the different contaminants.

There are several complications that must be recognized when generalizing about mixtures and coming from what we know about how single compounds act. First, a single compound may have multiple sites of action and these may be mediated by totally different mechanisms. Second, many substances, including metals, are changed to metabolites or conjugates in the body, and these new products may also have biologic activity that may or may not be similar to the parent compound. Thus even a single compound may become a functional mixture, as will be demonstrated for a single PCB congener and its hydroxylated metabolites. Third, there may be different effects of a single environmental contaminant at different ages. Lead is a clear example in that levels of blood lead that appear to have little effect on neurobehavioral function in an adult can cause an irreversible decrement in IQ and trigger altered behavior when impacting the developing nervous system in the prenatal or early postnatal period.

Environmental Diseases Resulting from Genetic Damage

As shown in Figure 2, there are extensive interactions among many of the various organ systems, such that alteration of one may influence the function of others. Central to many of the influences on biologic systems are effects that occur at the level of genes. Genes control almost everything, not just the eye color of our children. Cancer is a disease of genetic disruption. Cancer results from mutations in genes, some induced by a variety of environmental factors and some inherited from mutations in previous generations. Cancer genes may either be such as to promote generation of cancer (oncogenes) or, perhaps more frequently, are mutations that result in the loss of cancer suppressor functions. Many different environmental contaminants are carcinogenic, including some metals and organics. Mutations can cause birth defects, as normal development is under the control of genetics. But there can be other kinds of effects of environmental contaminants mediated by genetic dysfunction. During normal development genes are activated or inactivated at different stages, usually under the control of growth factors and hormones. Environmental contaminants may interfere with this developmental process. For example, many of the effects of diethylstilbestrol (DES), the estrogenic substance given to many pregnant women some years ago, were the result of altered expression of genes regulating sexual functions (*6*). Genes regulate many aspects of hormonal production, brain development and function, immune system balances, and organ physiology, as well as cancer and birth defects.

Environmental Contaminants and the Immune System

There are also many direct effects of environmental contaminants on various organ systems. The immune system, for example, is suppressed by some substances such as dioxin, coplanar PCBs, and PAHs (*7*). But the immune system is affected very differently by some metals, which promote hypersensitivity, rashes, and autoimmunity (*8*). An exciting developing area of investigation suggests that the dominance of different populations of T helper (Th) lymphocytes is a major factor in an individual's immune responsiveness. In individuals with normal immunity, it appears that the Th1 lymphocytes predominate; these lymphocytes produce a particular profile of cytokines. Individuals with hyperimmunity (showing asthma, skin rashes, and autoimmune syndromes) have predominately

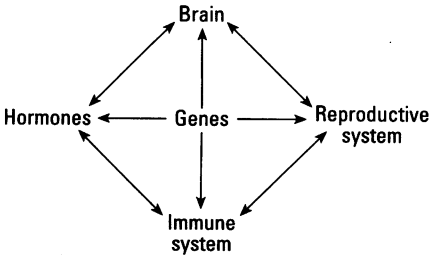

Figure 2. Diagrammatic interactions between genetic information and various organ systems intimately involved in the effects of environmental agents.

Th2 lymphocytes, which produce different cytokines (*9*). It has recently been demonstrated that environmental contaminants such as lead and mercury can alter the balance between the Th1 and Th2 lymphocytes (*10*) and there is speculation that contaminant exposure early in life may cause permanent or at least prolonged abnormalities in immune function.

The immune system is also intertwined with the other hormonal systems and the nervous system. Children exposed prenatally to DES show an altered immune function (*6*). There is also extensive interaction between the nervous and immune systems, even to the point of a common use of messengers. Although neurotransmitters and cytokines have traditionally been considered specific to only one system or the other, we now know that both are used at both sites (*11*).

Sex Steroids and the Nervous System

Estrogen has direct effects on neurons, such that estrogen alters synthesis of some transmitters (*12*), can alter neuronal structure (*13,14*), influences memory function (*15,16*), can trigger taste aversions (*17*), and can alter neuronal ionic currents (*18*). Furthermore, estrogens protect neurons against glutamate excitotoxicity (*19*).

Environmental Contaminants and the Nervous System

The nervous system is a frequent target of toxic action. A number of organic and inorganic compounds will cause abnormalities of peripheral sensory or motor nerves, resulting either in loss of sensation, abnormal sensation, or muscle weakness (*20*). Since the studies of Needleman and colleagues in 1979 (*21*), it has been accepted that lead at remarkably low concentrations can cause a decrement in IQ and also behavioral problems in children exposed prenatally and in the early postnatal years. More recent studies have suggested that these actions are irreversible (*22*). The exact mechanisms for these nervous system actions are not known. Evidence from several laboratories suggests that PCBs have similar effects; prenatal exposure has resulted in decrements in cognitive function and behavior (*23,24*) that appear irreversible (*25*).

Polychlorinated Biphenyls as Chemical Mixtures

Polychlorinated biphenyls are interesting compounds for illustration of the multiple effects of both individual chemicals and mixtures; throughout the rest of this paper, examples of results of PCB research from our group in Albany, New York, will be interposed. There are 209 possible PCBs and 75 dioxins, depending on the number and position of the chlorines on the base biphenyl or dioxin rings. PCBs were made as commercial mixtures with varying degrees of chlorination. Although their manufacture and use in the United States ceased in 1977 when they were recognized to be persistent both in the environment and in the body, they continued until recently to be manufactured and used in many countries of the world. Although they are persistent, they are altered by both physical and biologic processes because they are vulnerable to both anaerobic and aerobic biodegradation (*26–28*) and metabolism within the body (*29*). In most cases these various forms of metabolism alter the numbers and positions of the chlorines and do not totally degrade the PCB. Therefore, the number of different chemical compounds that can affect human and animal health is not limited to the approximately 150 congeners that were commercially produced.

Historically, PCBs have been considered weak dioxins. Indeed, some PCB congeners (those that can assume a coplanar configuration having chlorine atoms only in the *meta* and *para* positions to the biphenyl bridge) are weak activators of the aryl hydrocarbon (Ah) receptor, which is known to mediate many of the effects of 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), the most toxic polychlorinated dioxin congener (*30*). On the basis of this assumption, some have concluded that the PCBs that cannot assume a coplanar configuration are nontoxic (*31*), but this has now been proven incorrect. Results from our laboratories and those of a number of other researchers (*32–42*) clearly show that many of the 209 possible PCB congeners have discrete profiles of toxic actions and each congener may indeed have multiple actions at different sites.

ortho-substituted and lower chlorinated PCBs are neurotoxic and at least three different forms of direct neurotoxic actions have been demonstrated. Shain et al. (*32*) showed that congeners with two or more *ortho* chlorines inhibit the enzyme tyrosine hydroxylase, which is the rate-limiting enzyme for the synthesis of the neurotransmitter dopamine. Kodavanti et al. (*33*) and Carpenter et al. (*34*) showed that *ortho*-substituted, but not coplanar, congeners kill cerebellar granule cells by a mechanism that probably involves disruption of calcium homeostasis. Finally, Niemi et al. (*35*) demonstrated that both *ortho* and coplanar congeners are capable of blocking the process of long-term potentiation (LTP), an electrophysiologic measure in the brain that is thought to be correlated with cognitive function (*43*).

In addition to the direct effects of estrogens on neurons discussed above, other endocrine systems are important to nervous system function. One such system is the thyroid. The thyroid controls the rate of metabolism and is essential to organ development as well as daily function. Congenital hypothyroidism, even if treated after birth, results in a syndrome of minimal brain dysfunction (*44*). One end of the structure of the thyroid hormone shows some steric features similar to PCBs and dioxins and their hydroxylated metabolites. These substances interfere with normal thyroid function in a variety of ways, as outlined in Figure 3. Several

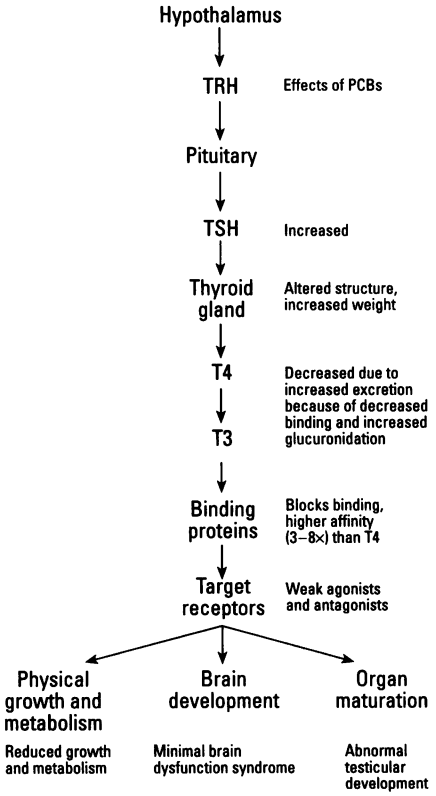

Figure 3. Diagrammatic indication of sites in which polychlorinated biphenyls alter thyroid hormone function and influence development. Abbreviations: T3, 3,3′,5′-triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyrotropin-stimulating hormone. Data from Porterfield (*44*), Byrne et al. (*45*), and Visser et al. (*46*).

studies have shown that PCBs cause hypothyroidism in animals (*45*), but there are a number of possible target sites that have been identified and it is likely that different congeners act at different sites (*46*). Some PCB congeners do not alter thyroid function (*36*) but the pattern of those that do and those that do not is not as simple as positioning the chlorines on the PCBs at sites comparable to those for the iodines on thyroid hormones.

Hypothyroidism results in reduced cognitive function and we have shown that animals made hypothyroid during postnatal development with the agent propylthiouracil (PTU) have a reduced LTP (*47*). Figure 4 shows results from an experiment in which we tested whether there was any interaction between the reduction of LTP induced directly by acute exposure to a PCB congener (2,4,4′-trichlorobiphenyl [TrCB]) and that caused by chronic PTU treatment. Clearly the effects are additive. In this experiment the hypothyroidism was not secondary to PCB exposure, but previous publications (*36,45–47*) document the fact that PCB exposure can cause hypothyroidism. This observation suggests an important principle when considering biologic responses to environmental contaminants: Even a single compound may influence a particular outcome by additive effects through totally different mechanisms. In this case PCBs may reduce LTP by causing hypothyroidism, but in addition PCBs may reduce LTP by a direct action such that the net effect may be additive.

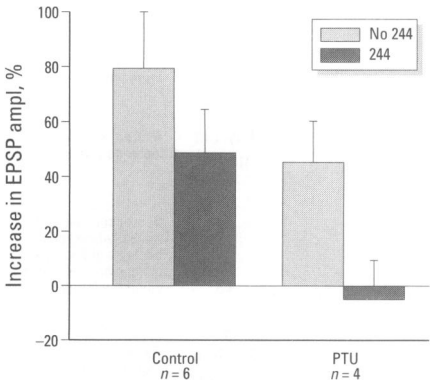

Figure 4. Additive effects of hypothyroidism induced by chronic treatment of developing animals with PTU, as described by Niemi et al. (*47*), and acute exposure of isolated hippocampal brain slices to 2,4,4′-TrCB on LTP, recorded in hippocampal area CA1, as described by Niemi et al. (*35*). Each bar represents the magnitude and SEM of the increase in the population excitatory postsynaptic potential (EPSP) induced by a tetanic activation of the synaptic input, which reflects LTP. Acute incubation of the brain slice with 1 µM 2,4,4′-TrCB resulted in about 50% reduction of LTP (right). Slices obtained from animals exposed to PTU postnatally, as described by Niemi et al. (*47*), also showed about 50% reduction in LTP as compared to control slices (no 244). When slices prepared from animals exposed to PTU were acutely incubated in the PCB congener, there was no LTP whatsoever (left, 244).

Endocrine disruption via interference with sex steroid hormones is a topic of intense interest, although it is not clear that these actions are necessarily more significant biologically than those actions that alter general metabolism via disruption of thyroid function. Many different chemicals show estrogenic, antiestrogenic, androgenic, or antiandrogenic activities (*37, 38,48–50*). Based to a great degree on the human experiments with DES (*6*) as well as on extensive information from wildlife (*51*), the sex steroid endocrine disruptive effects of xenobiotics have been suggested as causes of the reported decline in sperm count and general fertility (*3*), causes of birth defects of the reproductive system, contributors or causes of cancers of endocrine systems, and the causative agents for the perceived increase in alteration in sexual preferences (*52*). Further studies must be done, however, before it can be assumed that these conclusions apply to human populations.

Figure 5 shows the variety of ways in which xenobiotics can alter sex steroid function. Substances can mimic or antagonize endogenous hormones. They may alter the rates of synthesis or metabolism of the endogenous hormone or they may directly or indirectly alter the expression of receptors for the steroid (*53*). These various interactions may be complex in chemical mixtures, with each individual compound potentially having multiple actions and different compounds in the mixture potentially acting at different sites that influence the same final outcome.

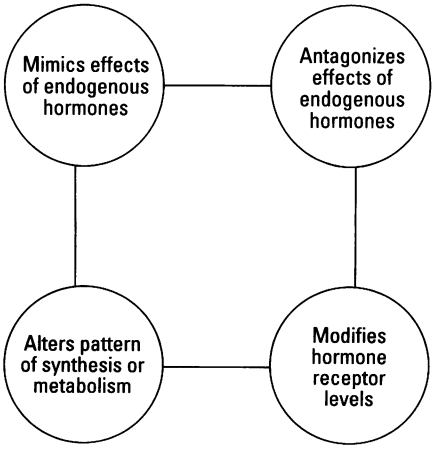

Figure 5. Ways in which different substances can cause endocrine disruption.

Figure 6 illustrates ways in which PCBs influence estrogenic function. Those coplanar PCBs that, like 2,3,7,8-TCDD, activate the Ah receptor, cause the induction of cytochrome P450s (CYP) of the *CYP1A* and *CYP1B* gene families. These P450s appear to catalyze the metabolism of many PCB congeners and other aromatic moieties such as endogenous hormones, including estradiol. They are all estradiol hydroxylases but insert the hydroxyl group at different sites: *CYP1A1* at the 2 position and *CYP1B1* at the 4 position (*54,55*). Estradiol can be oxidized at several positions, and the products are reactive, rapidly metabolized further, and excreted. Measurement of the 2- and 4-hydroxylated metabolites indicates the relative activity of the two forms of P450. When metabolism of estradiol is increased, functional levels fall and an altered estrogenic function ensues. A number of the *ortho*-substituted PCBs, but not the coplanars, produce a pattern of enzyme induction similar to that elicited by phenobarbital (*56*). Although the precise biochemical mechanisms and protein factors involved in this induction process are not well characterized, elevated levels of CYP2B, CYP2C, and CYP3A enzymes result and have the same effect in increasing metabolism and excretion. These enzymes are primarily expressed in the liver, although there may be limited expression in other tissues. Elevated rates of

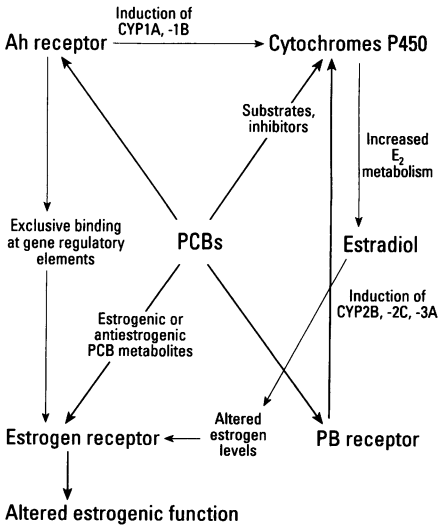

Figure 6. Sites of action of polychlorinated biphenyls in altering estrogenic function. PB, phenobarbital.

hepatic metabolism of estradiol are observed in animals exposed to PCBs (*57*). In contrast to CYP2B, CYP2C, and CYP3A enzymes, *CYP1A1* and *CYP1B1* appear to be inducible in a number of extrahepatic tissues including breast, uterus, and pituitary (*58,59*).

Some of the metabolites produced, especially mono- and dihydroxy PCBs, may have estrogenic or antiestrogenic activities of their own (*39*). In addition to inducing P450s, however, some PCBs can directly inhibit these enzymes. Although some lightly chlorinated PCBs bind to the active site of the P450s and hydroxylation of the compound occurs, some of the more heavily chlorinated congeners bind but are difficult to hydroxylate, so they are very effective inhibitors. Finally, through activation of the Ah receptor, 2,3,7,8-TCDD and probably also coplanar PCBs may have inhibitory effects on estrogen-regulated gene transcription by exclusive binding at gene regulatory elements found in the 5′ flanking regions of estrogen-responsive genes. The ligand-bound Ah receptor appears to disrupt the estrogen receptor–Sp 1 complex that is involved in transcriptional activation of human cathepsin D by interaction at an overlapping xenobiotic response element (*60*). There may be similar negative regulation of other estrogen-regulated genes by the Ah receptor.

Recent work in our laboratories using cultured human cells (*61*) has demonstrated how complex these interactions can be. Pang and co-workers measured CYP1A1 and CYP1B1 mRNA in several human cell lines including MCF-7 cells, a human breast cancer line (*40*). They demonstrated that a number of coplanar congeners increased both CYP1A1 and CYP1B1 mRNAs but *ortho*-substituted congeners did not. They then investigated estradiol metabolism by measuring levels of 2- and 4-methoxyestradiol, produced through the action of catechol-*O*-methyl transferase after estradiol is hydroxylated, by gas chromatography/mass spectrometry in the media of exposed cells. Although for some of the congeners there was a good correlation between the mRNA levels and the degree of estrogen metabolism, for others (especially 3,3′,4,4′,5,5′-hexachlorobiphenyl [HxCB] and 3,3′,4,4′,5-pentachlorobiphenyl [PeCB]), metabolism was much less than otherwise expected. This difference reflected a direct inhibition of the P450, which they showed by demonstrating that both of these congeners block the elevation of estradiol metabolism induced by 2,3,7,8-TCDD and by direct determination of inhibition of cDNA-expressed human CYP1B1 by 3,3′,4,4′,5,5′-HxCB in 3,3′,4,4′,5-PeCB. Thus these two congeners have opposing actions in this pathway—inducing mRNA for synthesis of an enzyme that they then directly inhibit.

Several other important conclusions come from this investigation. Previous studies of toxic equivalent factors (TEFs) of PCB congeners relative to 2,3,7,8-TCDD have been conducted primarily using rodents or rodent-derived cell lines. The values obtained from human cells are quite different, and the highest TEF is about an order of magnitude less than that derived from rodent studies. However, the different human cell lines also behave somewhat differently depending on which P450s they express. Thus the problem of extrapolation from animals to humans is complex. Also, the most potent PCB congener in stimulating estradiol metabolism in this study is 3,4,4′,5- tetrachlorobiphenyl (TeCB), an environmentally relevant congener that has not been previously identified as having high Ah receptor binding affinity nor assigned a TEF value (*62*).

Although most of the antiestrogenic actions of PCBs can be explained by effects on estrogen metabolism, there is also the clear possibility of action at estrogen receptors. Furthermore, the effects of PCB metabolites may be different from that of the parent compound. For example, Pang and co-workers (*40*) found that 3,4,5-TrCB was a potent inducer of CYP1A1 and CYP1B1 mRNA and a promoter of estrogen metabolism by both 2- and 4-hydroxylation. However, this compound is metabolized to a 4-biphenylol (Figure 7). Gierthy and colleagues (*39,63*) use the MCF-7 cell focus assay to identify estrogenic and antiestrogenic properties of xenobiotics; results with this parent compound and its metabolite are shown in Figure 8. In this assay, 3,4′,5-TrCB is antiestrogenic, probably as a result of induced metabolism of estradiol. However, the 4-hydroxy metabolite, 3,4′,5-TrCB-4-OH, has no antiestrogenic activity but shows clear estrogenic activity. Thus a parent compound and a metabolite may have diametrically opposite actions.

Figure 9 shows evidence that these processes occur in whole animals and again emphasizes how the different organ systems are interconnected. Compound 3,3′,4,4′-TeCB is a coplanar congener that is antiestrogenic (*37,38*). This congener has no effect on tyrosine hydroxylase activity (*32*). However, 3,3′,4,4′-TeCB is metabolized to a hydroxylated compound that is estrogenic. When developing rats are exposed to 2,2′,4,4′-TeCB, an *ortho*-substituted

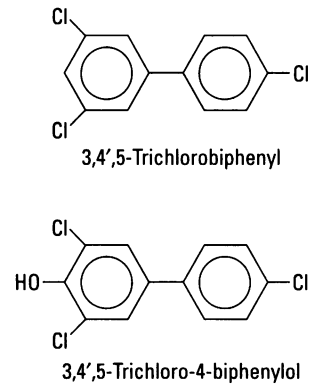

Figure 7. Structure of 3,4′,5-trichlorobiphenyl and its metabolic product 3,4′,5-trichloro-4-biphenylol.

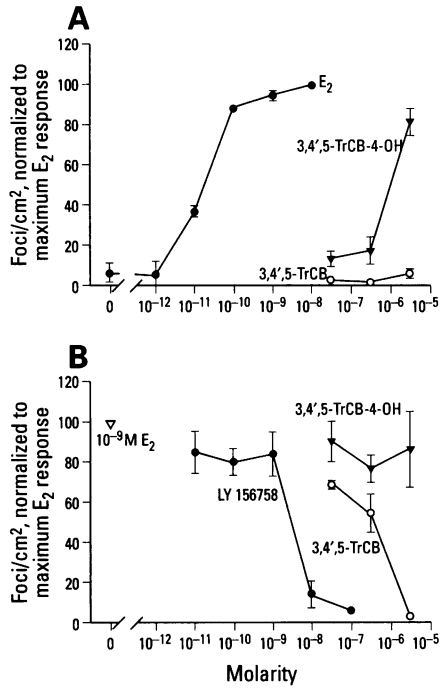

Figure 8. Estrogenicity and antiestrogenicity of 3,4′,5-trichlorobiphenyl and one of its metabolic products, tested in the MCF-7 cell focus assay as described by Gierthy et al. (*39,63*). (*A*) Dose–response relation for foci formation induced by E_2 and the biphenylol. Note the lack of estrogenic activity of the parent compound. 3,4′,5-trichloro-4-biphenylol is estrogenic at 5 μM and is thus 50,000 times less potent than estradiol. (*B*) Antiestrogenicity tested in the presence of 10^{-9} M E_2. The parent PCB is antiestrogenic, whereas the hydroxylated metabolic product shows no activity. Compared to the specific estrogen receptor blocker LY 156758, the parent PCB is approximately 100 times less potent.

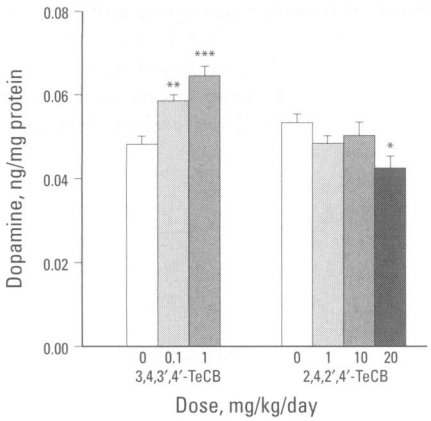

Figure 9. Dopamine concentrations in rat frontal cortex of animals exposed perinatally to various concentrations of 3,4,3′,4′- or 2,4,2′,4′ tetrachlorobiphenyl, as described by Seegal et al. (*41*). Note that although the *ortho*-substituted 2,4,2′,4′-TeCB resulted in a significant reduction in brain dopamine levels, the coplanar 3,4,3′,4′-TeCB caused a significant increase in dopamine levels. The former result is thought to be due to direct inhibition of the rate-limiting enzyme for dopamine synthesis (tyrosine hydroxylase), whereas the latter effect is a result of the activity of the hydroxylated degradation product, which is estrogenic. $*p < 0.05$, $**p < 0.01$, $***p < 0.01$.

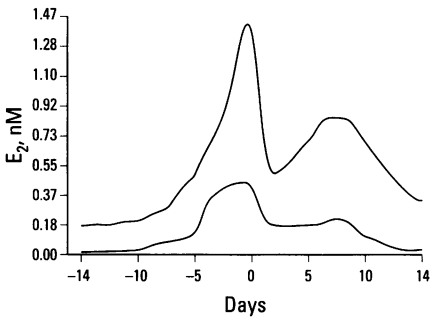

Figure 10. The normal variation of serum estrogen levels during the ovulatory cycle. The area between the upper and lower traces represents the range of E_2 profiles in normally ovulating women. Data derived from Diagnostic Products Corporation (*68*) and Thorneycroft et al. (*69*).

congener that inhibits tyrosine hydroxylase activity, there is a reduction in the level of brain dopamine in the adults. However, when animals are developmentally exposed to 3,3′,4,4′-TeCB, exactly the opposite result is found; dopamine levels are increased in adults, which may be due to the estrogenic activity of the hydroxylated metabolite of 3,3′,4,4′-TeCB (*41*).

The recent report by Arnold et al. (*64*) of synergistic actions of weak environmental estrogens has focused attention on the possibility that weakly active and especially persistent substances might have effects in combination that far exceed those expected by simple addition of effects. Although the results of this study have been questioned by its authors and others (*42,65,66*), there remains some evidence from behavioral studies that synergism does occur (*67*). Table 1 shows results from the study by Arnold et al. (*64*) (columns 2 and 3) as contrasted with those from Arcaro et al. (*42*) (columns 4 and 5), using the MCF-7 focus assay and a competitive receptor binding assay with purified recombinant human estrogen receptor (hER). Arnold et al. (*64*) used purified recombinant receptor directly (column 3) or expressed this receptor in yeast together with the β-galactosidase (β-gal) reporter gene (column 2) to test the estrogenlike activity of hydroxylated PCBs and pesticides. Results in column 2 and 3 show that both the EC_{50} for β-gal activity and the IC_{50} for binding the hER are significantly lower for a combination of the two hydroxylated PCBs than for either compound alone, indicating that in combination the hydroxylated PCBs are significantly more potent. Similar conclusions were drawn for the pesticides dieldrin and endosulfan. In the study by Arcaro et al. (*42*), in both the MCF-7 cell focus assay and the hER binding assay, hydroxylated PCBs alone showed estrogenlike activity. However, mixtures were not more potent, indicating that no synergy occurred. Of the pesticides studied, only endosulfan was weakly estrogenic and the combination with dieldrin was not synergistic.

The most important question with regard to weak environmental estrogens is whether they interact with endogenous estradiol. This is an important question not only for women of reproductive age, who have high and fluctuating estrogen levels, but especially for children, postmenopausal women, and men, whose estrogen levels are low. Figure 10 illustrates the typical fluctuations of estradiol concentration during the ovulatory cycle. Figure 11 shows results of the estrogenic response in MCF-7 cells to estradiol alone, tested at 10^{-12} to 10^{-8} M, and with three different concentrations of the estrogenic PCB metabolite 2,4,6-TrCB-4′-OH. It is important to note that there is no evidence of any synergistic effect of estradiol and 2,4,6-TrCB-4′-OH on the response of MCF-7 cells. We conclude that different amounts of this estrogenic PCB metabolite together with varying physiologic concentrations of estradiol do not exhibit any synergism in the *in vitro* situation. However, this *in vitro* study does not negate the synergism study in turtles (*67*), although it does pose a challenge for building a stronger case that synergism between environmentally relevant estrogenic substances occurs in humans.

In summary, it is difficult to study chemical mixtures because of the variety of ways in which the components may interact. However, even single compounds can have complex actions at multiple sites, varying with stage of development, and

Table 1. Estrogenic and synergistic effects of weak xenoestrogens and mixtures.

Chemical	β-gal activity, EC_{50} μM[a]	hER binding, IC_{50} μM[a]	MCF-7 focus development, EC_{50} μM[b]	hER binding[2] IC_{50} μM[b]
17β-estradiol	0.0001	0.001	0.0003	0.0005
2,4,6-TCB-4′-OH	0.0070	0.055	0.22	0.079
2,3,4,5′-TCB-4′-OH	0.0180	0.12	0.72	0.015
2,4,6-TCB-4-OH and 2,3,4,5-TCB-4′-OH	0.0015	0.005	0.18	0.015
Dieldrin	>33	>50	ND	ND
Endosulfan	>33	>50	>10	ND
Dieldrin and endosulfan	0.092	0.324	>10	ND

Abbreviations; EC_{50}, median effective concentration; IC_{50}, concentration that inhibits 50%; ND, not detectable. [a]Data from Arnold et al. (*64*). [b]Data from Arcaro et al. (*42*).

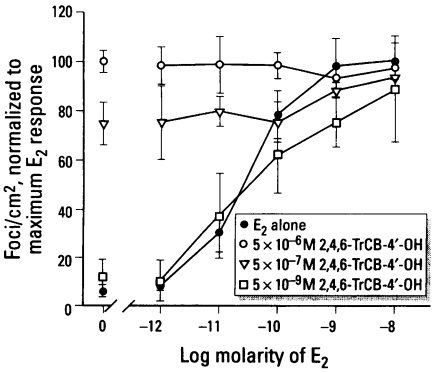

Figure 11. The estrogenic effects of three concentrations of 2,4,6-trichloro-4′-biphenylol alone and together with dose–response curves of E_2 in the MCF-7 cell focus assay, as described by Arcaro et al. (*42*). The data show no synergistic effect when the biphenylol at various concentrations was added together with E_2.

may become functional mixtures in the body as a result of metabolism. In reality people are exposed to mixtures and if we are ever to understand the human diseases that people develop from exposure to environmental contaminants, we must study and understand the interactions that occur in mixtures. At the same time, it will probably not be possible to understand the complexity of mixtures without understanding the mechanisms whereby individual contaminants and their metabolites act, recognizing all of the problems associated with species and organ specificities, age-dependent actions, dose–response relationships, and the enormous interdependence of the various organ systems that can lead to indirect as well as direct effects.

REFERENCES AND NOTES

1. Bush B, Seegal RF, Fitzgerald E. Human monitoring of PCB urine analysis. In: Organohalogen Compounds, Vol 1: Dioxin '90—EPRI Seminar, Toxicology, Environment, Food, Exposure-Risk (Hutzinger O, Fiedler H, eds). Bayreuth:Ecoinforma Press, 1990;509–513.
2. ATSDR. Priority Health Conditions. An Integrated Strategy to Evaluate the Relationship between Illness and Exposure to Hazardous Substances (Lybarger JA, Spengler RF, DeRosa CT, eds). Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1993.
3. Sharpe RM, Shakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 341:1392–1395 (1993).
4. Emmerson BT. Chronic lead nephropathy. Kidney Int 4:1–5 (1973).
5. Clarkson TW. Methylmercury toxicity to the mature and developing nervous system: possible mechanism. In: Biological Aspects of Metals and Metal-related Diseases (Sarkar B, ed). New York:Raven Press, 1983;183–197.
6. Newbold R. Cellular and molecular effects of developmental exposure to diethylstilbestrol: implications for other environmental estrogens. Environ Health Perspect 103(Suppl 7):83–87 (1996).
7. Kerkvliet NI, Steppan LB, Brauner JA, Deyo JA, Henderson MC, Tomar RS, Buhler DR. Influence of the Ah locus on the humoral immunotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: evidence for Ah-receptor-dependent and Ah-receptor-independent mechanisms of immunosuppression. Toxicol Appl Pharmacol 105:26–36 (1990).
8. Waterman SJ, El-Fawal HAN, Snyder CA. Lead alters the immunogenicity of two neural proteins: a potential mechanism for the progression of lead-induced neurotoxicity. Environ Health Perspect 102:1052–1056 (1994).
9. Swain SL, Bradley LM, Croft M, Tonkonogy S, Atkins G, Weinberg AD, Duncan DD, Hedrick SM, Dutton RW, Huston G. Helper T-cell subsets: phenotype, function and role of lymphokines in regulating their development. Immunol Rev 123:115–144 (1991).
10. Heo Y, Parsons PJ, Lawrence DA. Lead differentially modifies cytokine production *in vitro* and *in vivo*. Toxicol Appl Pharmacol 138:149–157 (1996).
11. Dafny N, Prieto-Gomez B, Reyes-Vasquez C. Does the immune system communicate with the central nervous system? J Neuroimmunol 9:1–12 (1985).
12. Pasqualini C, Olivier V, Guibert B, Frain O, Leviel V. Acute stimulatory effect of estradiol on striatal dopamine synthesis. J Neurochem 65:1651–1657 (1993).
13. Kawata M. Roles of steroid hormones and their receptors in structural organization in the nervous system. Neurosci Res 24:1–46 (1995).
14. Woolley CS, McEwen BS. Estradiol regulates hippocampal dendritic spine density via an *N*-methyl-D-aspartate receptor-dependent mechanism. J Neurosci 14:7680–7687 (1994).
15. Kampen DL, Sherwin BB. Estradiol is related to visual memory in healthy young men. Behav Neurosci 110:613–617 (1996).
16. Luine V, Roderiguez M. Effects of estradiol on radial arm maze performance of young and aged rats. Behav Neural Biol 62:230–236 (1994).
17. Ganesan R, Simpkins JW. Conditioned taste aversion induced by estradiol pellets. Physiol Behav 50:849–852 (1991).
18. Mermelstein PG, Becker JB, Surmeier DJ. Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. J Neurosci 16:595–604 (1996).
19. Singer CA, Rogers KL, Strickland TM, Dorsa DM. Estrogen protects primary cortical neurons from glutamate toxicity. Neurosci Lett 212:13–16 (1996).
20. Herskowitz A, Ishii N, Schaumburg H. *n*-Hexane neuropathy. N Engl J Med 285:82–85 (1971).
21. Needleman HL, Gunnoe C, Leviton A, Reed R, Peresie H, Maher C, Barrett P. Deficit in psychologic and classroom performance of children with elevated dentine lead levels. N Engl J Med 300:689–695 (1979).
22. Needleman HL, Schell A, Bellinger D, Leviton A, Alfred EN. The long-term effects of exposure to low doses of lead in childhood: an 11-year follow-up report. N Engl J Med 322:83–88 (1990).
23. Rogan WJ, Gladen BC. Neurotoxicology of PCBs and related compounds. Neurotoxicology 13:27–36 (1992).
24. Yu M-L, Hsu C-C, Guo YL, Lai T-J, Chen S-J, Luo, J-M. Disordered behavior in the early-born Taiwan YuCheng children. Chemosphere 29:2413–2422 (1994).
25. Jacobson JL, Jacobson SW. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. N Engl J Med 355:783–789 (1996).
26. Quensen JF, Tiedje JM, Boyd SA. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242:752–754 (1988).
27. Rhee G-Y, Sokol RC. The fate of polychlorinated biphenyls in aquatic sediments. Great Lakes Res Rev 1:23–28 (1994).
28. Sokol RC, Kwon O, Bethoney CM, Rhee G-Y. Reductive dechlorination of polychlorinated biphenyls in St. Lawrence River sediments and variations in dechlorination characteristics. Environ Sci Technol 28:2054–2064 (1994).
29. Phillips DL, Smith AB, Burse VW, Steele GK, Needham LL, Hannon WH. Half-life of polychlorinated biphenyls in occupationally exposed workers. Arch Environ Health 44:351–354.
30. Ahlborg UG, Beching GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, et al. Toxic equivalency factors for dioxin-like PCBs. Chemosphere 2:1049–1067 (1994).
31. Abramowicz DA. Aerobic and anaerobic biodegradation of PCBs: a review. Crit Rev Biotech 10:241–251 (1990).
32. Shain W, Bush B, Seegal R. Neurotoxicity of polychlorinated biphenyls: structure-activity relationship of individual congeners. Toxicol Appl Pharmacol 111:33–42 (1991).
33. Kodavanti PRS, Shin D-S, Tilson HA, Harry GJ. Comparative effects of two polychlorinated biphenyl congeners on calcium homeostasis in rat cerebellar granule cells. Toxicol Appl Pharmacol 123:97–106 (1993).
34. Carpenter DO, Stoner CRT, Lawrence DA. Flow cytometric measurements of neuronal death triggered by PCBs. Neurotoxicology 18:507–514 (1997).
35. Niemi WD, Audi J, Bush B, Carpenter DO. PCBs reduce long-term potentiation in the CA1 region of rat hippocampus. Exp Neurol 151:26–34 (1998).
36. Ness DK, Schantz SL, Moshtaghian J, Hansen LG. Effects of

perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. Toxicol Lett 68:311–323 (1993).
37. Krishnan V, Safe S. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. Toxicol Appl Pharmacol 120:55–61 (1993).
38. Jansen HT, Cooke PS, Porcell J, Liu TC, Hansen LG. Estrogenic and antiestrogenic cations of PCBs in the female rat: *in vitro* and *in vivo* studies. Reprod Toxicol 7:237–248 (1993).
39. Gierthy JF, Arcaro KF, Floyd M. Assessment of PCB estrogenicity in a human breast cancer cell line. Chemosphere 34:1495–1505 (1997).
40. Pang S, Cao JQ, Katz BH, Hayes CL, Sutter TR, Spink DC. Inductive and inhibitory effects of non-*ortho*-substituted polychlorinated biphenyls on estrogen metabolism and human cytochromes P450 1A1 and 1B1. Biochem Pharmacol (in press).
41. Seegal RF, Brosch KO, Okoniewski RJ. Effects of *in utero* and lactational exposure of the laboratory rat to 2,4,2′,4′- and 3,4,3′,4′-tetrachlorobiphenyl on dopamine function. Toxicol Appl Pharmacol 146:95–103 (1997).
42. Arcaro KF, Vakharia DD, Yang Y, Gierthy JF. Lack of synergy of weakly estrogenic hydroxylated polychlorinated biphenyls and pesticides. Environ Health Perspect 106(Suppl 4):1041–1046 (1998).
43. Gomez RA, Passo Miller LD, Aoki A, Ramirez OA. Long-term potentiation-induced synaptic changes in hippocampal *dentate gyrus* of rats with an inborn low or high learning capacity. Brain Res 537:293–297 (1990).
44. Porterfield SP. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ Health Perspect 102(Suppl 2):125–130 (1994).
45. Byrne JJ, Carbone JP, Hanson EA. Hypothyroidism and abnormalities in the kinetics of thyroid hormone metabolism in rats treated chronically with polychlorinated biphenyl and polybrominated biphenyl. Endocrinology 121:520–527 (1987).
46. Visser TJ, Kaptein E, van Toor H, van Raaij JAGM, van den Berg KJ, Joe CTT, van Engelen JGM, Brauwer A. Glucuronidation of thyroid hormone in rat liver: effects of *in vivo* treatment with microsomal enzyme inducers and *in vitro* assay conditions. Endocrinology 133:2177–2186 (1993).
47. Niemi WD, Slivinski K, Audi J, Rej R, Carpenter DO. Propylthiouracil treatment reduces long-term potentiation in area CA1 of neonatal rat hippocampus. Neurosci Lett 210:127–129 (1996).
48. Spink DC, Lincoln DW, Dickerman HW, Gierthy JF. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. Proc Natl Acad Sci USA 87:6917–6921 (1990).
49. Gierthy JF, Spink BC, Figge HL, Pentecost BT, Spink DC. Effects of 2,3,7,8-tetrachlorobidenzo-*p*-dioxin, 12-*O*-tetradecanoylphorbol-13-acetate and 17β-estradiol on estrogen receptor regulation in MCF-7 human breast cancer cells. J Clin Biochem 60:173–184 (1996).
50. Li MH, Hansen LG. Enzyme induction and acute endocrine effects in prepubertal female rats receiving environmental PCB/PXDF/PCDD mixtures. Environ Health Perspect 194:712–722 (1996).
51. Colborn T, vam Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect 101:378–384 (1993).
52. Colborn T, Dumanoski K, Myers JP. Our Stolen Future: Are We Threatening Our Fertility, Intelligence and Survival? A Scientific Detective Story. New York:Dutton, 1996.
53. Flouriot C, Pakdel F, DuCouret B, Valotaire Y. Influence of xenobiotics on rainbow trout liver estrogen receptor and vitellegenin gene expression. J Mol Endocrinol 15:143–151 (1995).
54. Spink DC, Hayes CL, Young NR, Christou M, Sutter TR, Jefcoate CR, Gierthy JF. The effects of 2,3,7,8-tetrachlorobidenzo-*p*-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17β-estradiol 4-hydroxylase. J Steroid Biochem Mol Biol 51:251–258 (1994).
55. Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR. 17β-estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc Natl Acad Sci USA 93:9776–9781 (1996).
56. Parkinson A, Safe S, Robertson LW, Thomas PE, Ryan DE, Reik LM, Levin W. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships. J Biol Chem 258:5967–5976 (1983).
57. Namkung MJ, Porubek DJ, Nelson SD, Juchau MR. Regulation of aromatic oxidation of estradiol-17β in maternal hepatic, fetal hepatic and placental tissues: comparative effects of a series of inducing agents. J Steroid Biochem 22:563–567 (1995).
58. Guengerich FP. Characterization of human microsomal cytochrome P450 enzymes. Annu Rev Pharmacol Toxicol 29:241–264 (1989).
59. Shimada T, Hayes CL, Yamazaki H, Amin S, Hect SS, Guengerich FP. Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. Cancer Res 56:2979–2984 (1996).
60. Krishnan V, Porter W, Santostefano M, Wang X, Safe S. Molecular mechanism of inhibition of estrogen-induced cathepsin D gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in MCF-7 cells. Mol Cell Biol 15:6710–6719 (1995).
61. Pang S. Development and application of HPLC/MS and GC/MS methodologies to determine the effect of polychlorinated aromatic compounds on estrogen metabolism in human-derived cell lines [PhD Dissertation]. The University at Albany School of Public Health, Albany, NY, 1997.
62. Safe S. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24:87–149 (1994).
63. Gierthy JF, Lincoln DW, Roth KE, Bowser SS, Bennett JA, Bradley L, Dickerman HW. Estrogen-stimulation of postconfluent cell accumulation and foci formation of human MCF-7 breast cancer cells. J Cell Biochem 45:177–187 (1991).
64. Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ Jr, McLachlan JA. Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272:1489–1491 (1996).
65. McLachlan JA. Synergistic effect of environmental estrogens: report withdrawn. Science 277:462–463 (1997).
66. Ramamoorthy K, Wang F, Chen I-C, Norris JD, McDonnell DP, Leonard LS, Gaido KW, Bocchinfuso WP, Korach KS, Safe S. Estrogenic activity of a dieldrin/toxaphene mixture in the mouse uterus, MCF-7 human breast cancer cells, and yeast-based estrogen receptor assays: no apparent synergism. Endocrinology 138:1520–1527 (1997).
67. Bergeron JM, Crews D, McLachlan JA. PCBs as environmental estrogens: turtle sex determination as a biomarker of environmental contamination. Environ Health Perspect 102:780–781 (1994).
68. Diagnostic Products Corporation. Publ PITK E2-F. Los Angeles, CA:Diagnostic Products Corp., 1995
69. Thorneycroft IH, Mishell DR Jr, Stone SC, Kharma KM, Nakamura RM. The relation of serum 17-hydroxyprogesterone and estradiol-17β levels during the human menstrual cycle. Am J Obstet Gynecol 111:947–951 (1971).