Understanding the Human Health Effects of Chemical Mixtures

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Most research on the effects of chemicals on biologic systems is conducted on one chemical at a time. However, in the real world people are exposed to mixtures, not single chemicals. Although various substances may have totally independent actions, in many cases two substances may act at the same site in ways that can be either additive or nonadditive. Many even more complex interactions may occur if two chemicals act at different but related targets. In the extreme case there may be synergistic effects, in which case the effects of two substances together are greater than the sum of either effect alone. In reality, most persons are exposed to many chemicals, not just one or two, and therefore the effects of a chemical mixture are extremely complex and may differ for each mixture depending on the chemical composition. This complexity is a major reason why mixtures have not been well studied. In this review we attempt to illustrate some of the principles and approaches that can be used to study effects of mixtures. By the nature of the state of the science, this discussion is more a presentation of what we do not know than of what we do know about mixtures. We approach the study of mixtures at three levels, using specific examples. First, we discuss several human diseases in relation to a variety of environmental agents believed to influence the development and progression of the disease. We present results of selected cellular and animal studies in which simple mixtures have been investigated. Finally, we discuss some of the effects of mixtures at a molecular level. *Key words:* **aromatic hydrocarbon receptor, cancer, cardiovascular disease, endocrine disruptors, metals, neurobehavioral abnormalities, neurodegenerative diseases, PAHs, PCBs, synergy.** *Environ Health Perspect* **110(suppl 1):25–42 (2002).**
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Assessing Health Effects of Chemical Mixtures

There is no question that many chemicals cause human disease. Arsenic and skin cancer, asbestos and lung cancer, lead and decrements of IQ, and dioxin and chloracne are examples of well-documented effects. However, most people are not exposed to only a single chemical compound. Although the health effects of single contaminants may be apparent under circumstances of high exposure, the great majority of people are exposed to chemical mixtures of organics and inorganics at lower concentrations. And though each of these contaminants individually may increase risk of certain diseases, the question of how contaminants interact remains a relatively unexplored subject but one recently recognized for its increasing importance (*1–4*).

The study of chemical mixtures is limited for a number of reasons. It is much easier to study a single compound in an animal study and to obtain traditional dose–response information. An almost infinite number of combinations of contaminants is possible, and often we do not know which is most important, or which dose ranges should be investigated, or which biologic end points should be studied. Although relatively few studies have investigated the interactions of even two chemicals, in real life we are all exposed to multiple substances, and the biologic effects of 20 different chemicals may be very different from those of just two. Furthermore, even the statistics relating to how one deals with complex mixtures is a newly developing science (*2*).

The number of chemicals to which humans are exposed has increased dramatically in the past 100 years. Mankind has always been exposed to various metals, which as natural elements are present throughout the environment, in drinking water, and in food. Many natural chemicals are in the foods that we eat, and many of these act at a variety of sites in different organs and cells. Polycyclic aromatic hydrocarbons (PAHs), formed by combustion, have been a source of exposure since humans learned to produce fire. With the development of the use of fossil fuels for many purposes, humans have become exposed to a greater range of hydrocarbons and their byproducts. But the number of chemicals produced by the chemical and pharmaceutical industries in the twentieth century has vastly increased human exposure. We produce almost all food crops through use of pesticides, herbicides, and fungicides. We produce meat products through extensive use of growth stimulants and antibiotics. The rapid development in use of plastics has resulted in exposure to various chemicals that may leach into food. The number of medicines has increased enormously, most of which are clearly of benefit to human health but still have the possibility of interactions with other environmental agents. Pimentel et al. (*5*) report that some 80,000 chemicals are in use today, that nearly 10% are recognized as carcinogens, and that use of chemicals has increased 3-fold from 1941 to 1995. Many of these compounds have not been adequately tested for human toxicity. For example, the National Toxicology Program has published only 605 reports of long- or short-term study of chemicals, a very small proportion of those in use today.

Interactions of Chemicals in Biologic Systems

Chemicals can interact in a number of ways. If we consider two chemicals, they may act at a common site such as a receptor or an enzyme. In this case their actions may be additive if both activate the target, or occlusive if one activates and the other binds without activating or binds with a slow dissociation constant. However, many effects are more complex than simply binding to a receptor or enzyme and act through some combination of altering gene expression, changing levels of intracellular concentrations of ions, altering cellular metabolism or production of cellular regulators. Under these circumstances the effect of mixtures is more difficult to predict. In reality, few chemicals have only a single cellular target. Most act at multiple sites on different cell types or in some cases even at multiple targets within the same cell type. There may be quite different actions on the kidney, the liver, and the brain, each with a different disease-related outcome. The actions at each of these sites depend on the presence of genes, receptors, and cellular regulators in the specific cell types. When targets that regulate other organs and cells are affected (e.g., the thyroid or the beta cells of the pancreas), the impact of the chemical agent is much greater.

Much of the contemporary concern about chemical mixtures stems from the possibility of compounds having synergistic, or more than additive, effects. We have a few

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examples of well-documented synergistic actions of environmental agents in humans. We have strong evidence that inhalation of radon progeny and current smoking have synergistic effects on the incidence of lung cancer (*6*). We even have evidence that quitting smoking decreases lung cancer risk from radon more than reduction of radon exposure (*7*). We also have strong evidence that smoking and exposure to asbestos exert synergistic effects on incidence of lung cancer [see Erren et al. (*8*)]. Obviously smoking, considered alone, represents an exposure to a very complex chemical mixture, but this does not alter the clear evidence that the addition of a second environmental factor, either radon or asbestos, results in more than an additive risk of injury. However, we have little additional documentation of how mixtures in humans interact, and little investigation of this question even in animals.

Routes of Exposure and Exposure Assessment

Exposure to chemical mixtures may result from ingestion, inhalation, or dermal absorption. Often the route of exposure defines the site of disease. For example, smoking causes lung cancer, and mutagenic substances applied to the skin cause skin cancer. Some contaminants may induce differential effects depending on the route of exposure—for example, asbestos inhaled versus asbestos ingested. This is understandable because site of application reflects local mutagenic effects or generation of reactive substances, such as reactive oxygen species (ROS; discussed below). The body rapidly absorbs and distributes other toxicants regardless of the route of exposure, and the health outcomes are not specific to route of exposure. For the more persistent contaminants, the route of exposure is less significant because they are present long enough to equilibrate in the body. Because different kinds of contaminants may have different reservoirs in the body (e.g., metals to bone and teeth, organics to adipose tissue), toxic actions in the reservoirs may also be predictive of disease there. However, each of these reservoirs is usually in equilibrium with levels in blood and tissues.

A full discussion of exposure assessment is beyond the scope of this review because it is one of the most difficult problems facing environmental health scientists. Some substances are persistent in the body, and direct measurement of concentration in body fluids or tissues provides information that allows one to estimate exposure. However, even these cases almost always involve some metabolism and/or excretion, so determining the degree of exposure in the past is difficult. On the other hand, many other toxic substances are not persistent and may exert harmful effects that last long after the agent is no longer present in the body. Assessing exposure to these substances is more difficult. Some do leave biomarkers of exposure, such as DNA or protein adducts, and study of such adducts is an active area of investigation. Many carcinogenic substances induce gene mutations as the primary event, activating oncogenes or inactivating tumor suppressor genes, and such mutations can be monitored as biomarkers of exposure (*9*). Therefore, all too often the environmental health scientist is left with only interview reports of exposure, occupational history, or other less rigorous evidence from which to draw conclusions about relationship to disease.

One of the few standard methods for dealing with exposure assessment of mixtures has been the use of toxic equivalent factors (TEFs) for dioxinlike substances (*10*). Because dioxins, furans, some polychlorinated biphenyls (PCBs), and some PAHs all act at the aromatic hydrocarbon receptor (AhR) to induce a profile of effects (many of them adverse), assays that determine the degree of AhR activation are often used to evaluate mixture toxicity (*11*). This approach assumes that all substances acting at the AhR produce the same profile of effects and that the actions are additive. Although such an approach has clear value in determining the toxicity of a complex mixture, it also has some major limitations. Different species differ in potency, with values usually derived from short-term, *in vitro* studies that ignore the toxicokinetics (*1*). Wölfle (*12*) has shown that polychlorinated dibenzodioxins (PCDDs) plus PCB 126 have additive effects, as we might predict, whereas PCB 153, a di-*ortho* congener that does not strongly activate the AhR, antagonized the PCDD action. AhR activation does not mediate many effects of PCBs. Because of the widespread acceptance of the use of TEFs for dioxin toxicity, many people ignore the non–dioxin-like effects of such agents as the noncoplanar PCBs. These other PCBs may not be as carcinogenic as the coplanar ones, but the health outcomes from these other congeners (neurobehavioral abnormalities, endocrine and metabolic disruption) may in fact be of greater overall importance than cancer. Other assays have proven valuable for analysis of other parameters of mixtures, such as estrogenic activity (*13,14*), but none of these has utility for mixtures of organics and metals or other combinations for which the initial action is not at a common site.

Environmental Contributions to Human Disease

A critical first question in evaluating the role of chemical mixtures in human disease is to discuss the degree to which environmental agents contribute to disease in general. Even on this issue, there is no widespread consensus. Murray and Lopez (*15*) have estimated that environmental factors (chemicals, radiation, and tobacco smoke together) are responsible for roughly 80% of all cancers. Smith et al. (*16*) have estimated that 25–33% of the total global burden of disease can be attributed to environmental factors and that children are particularly vulnerable to environmental factors. This estimate includes diseases secondary to infectious agents transmitted through the environment. In recent years, evidence has emerged for a contribution of environmental agents to a number of diseases that have not previously been considered to have environmental causes. This is especially true for the general categories of diseases considered under the rubric of endocrine disruption, as well as a number of chronic diseases such as cardiovascular disease (see below), diabetes (*17–19*), and even bone, joint, and intervertebral disk disease (*20*). In many of these situations we have some evidence for a variety of chemicals having a relationship to the disease, but we currently have little or no evidence of the nature and effects of interactions among chemicals related to the disease state.

We can divide the diseases that we suspect have a relationship to environmental agents into two broad categories: those agents that interfere with normal development and distort physiologic function and those that cause direct cellular damage. Although it is not possible to discuss in this review all the human diseases that environmental chemicals influence, and although we have little or no rigorous information on the actions of chemical mixtures for most of these diseases, we use several diseases in each category as examples of the present state of our knowledge and the research questions that need to be addressed.

Human Diseases Based on Current Understanding of Physiologic Mechanisms

Many environmental agents act to alter the biochemistry and physiology of the organism, but they do so without necessarily shortening life or even causing overt disease. If these actions occur during development, they can result in permanent, life-long differences in physical size, intelligence, behavior, reproductive ability, and susceptibility to other diseases. Even later in life such exposures may alter mental and sexual functions. Many use the term "endocrine disruption" to encompass these different effects, but this term is inadequate to include the full range of alterations that may result in

organs, such as the brain, that are not secondary to changes in the endocrine systems. Furthermore, some of the changes in physiologic function may not, when considered alone, result in cellular damage, but they may increase risk of disease, as described below for cardiovascular disease in relation to factors that increase serum lipids. We describe two categories of disease below to illustrate these points.

Neurobehavioral Abnormalities

In 1979 Needleman et al. (*21*) first reported clear evidence that exposure of young children to lead resulted in both a decrement in IQ and the development of a series of disruptive behaviors characterized by a shortened attention span. They followed these children for a number of years, and later Needleman et al. (*22*) concluded that the effects of lead on neurobehavior function were essentially irreversible, in that the decrements did not diminish with time. Consistent with this conclusion is the observation of Rogan et al. (*23*) that lead chelation reduces blood lead levels in children over 2–3 years old but will not reverse the neurocognitive deficits. Although the IQ deficits were only in the range of 5–7 IQ points and therefore probably do not result in enormous differences in individual performance, from a societal perspective, a systematic "dumbing down" of the population has enormous consequences.

More recently it has become clear that several organic pollutants have similar effects. The Yucheng cohort in Taiwan had prenatal exposures to a mixture of PCBs and furans, and these children showed a similar 5- to 7-point IQ deficit that did not disappear with time (*24*). These children also showed disordered and mildly antisocial behavior (*25*), which is very similar to that described by Needleman et al. (*21*) in their initial lead study. Neurobehavioral decrements have been confirmed in several populations in the United States exposed to PCBs through consumption of contaminated fish (*26,27*) or general diet (*28*), although in all these studies the PCBs were not the only contaminant to which subjects had been exposed. Fish, in particular, often contain significant amounts of methyl mercury, which also has significant neurobehavioral effects in humans at very high exposure levels (*29*). Significant controversy remains as to whether methyl mercury, at the concentrations accumulated in populations that consume a large amount of fish and marine mammals, causes neurobehavioral damage. Davidson et al. (*30*) have studied the population of the Seychelles, where levels of methyl mercury are high, and found—if anything—superior performance in those children with higher methyl mercury levels, which they attribute to the beneficial effects of fish consumption. On the other hand, Grandjean et al. (*31*) studied the population of the Faroe Islands, who also eat a lot of fish, and reported significant decrements of IQ and abnormalities of behavior in children that increase with exposure levels. We have some evidence that adults who eat a significant amount of contaminated fish suffer from some cognitive deficits, attributed in different regions to methyl mercury by Mergler et al. (*32*) and to PCBs by Schantz et al. (*33*). One major question is whether it is the methyl mercury or the PCBs, both of which are present in most situations, that cause these effects, or whether perhaps it is the combination of the two. A recent report by Bemis and Seegal (*34*) provides some evidence for a synergistic interaction between methyl mercury and PCBs in reducing the content of the neurotransmitter dopamine from brain.

Pesticide exposure has also been reported to have adverse effects on neurobehavioral functioning. Guillette et al. (*35*) studied children of two ethnic minority communities in Mexico: in one community the people lived and worked on a plain with heavy agriculture using a variety of pesticides; the other community was in the foothills, which had little or no agriculture. The children exposed to pesticides had significantly less ability to draw a human form, and they demonstrated a variety of motor deficits for simple tasks.

Animal studies have shown decrements in ability to learn as a result of exposure to lead (*36*), PCBs (*37*), dioxins (*38*), and organophosphate pesticides (*39*). However, with the notable exception of the study of Bemis and Seegal (*34*) (which was not a study of learning), there has been no systematic study of mixtures of lead, methyl mercury, PCBs, furans, dioxins, or various pesticides on learning and memory, either in humans or in animals. We do not well understand the mechanisms by which these substances cause neurobehavioral effects. Although disruption of some endocrine systems, especially thyroid hormone, can result in neurobehavioral decrements, and although several xenobiotics alter thyroid function (*40*), it is clear that lead and PCBs, at least, have a direct effect on nervous system function that is not mediated by alteration of thyroid hormone. Lead (*41*) and PCBs (*42*) both block the process of long-term potentiation, a form of synaptic plasticity that has often been used as a model system for study of cognitive potential (*43*). These actions occur both with chronic perinatal exposure of the developing animal and with acute *in vitro* application of the toxicant. Experimental hypothyroidism also reduces long-term potentiation (*44*). We urgently need to study the effects of mixtures of metals, PCBs, and pesticides on neurobehavioral function, both their direct actions on the brain and their interactions with altered endocrine systems, especially thyroid function.

Sex Steroid Hormonal Disruption

The developing organism is exquisitely sensitive to alterations in hormone function. In the early embryonic state, the gonads of human males and females are morphologically identical. Sexual differentiation begins under hormonal influence during the fifth and sixth weeks of fetal development, and thus alteration of hormone function during this highly sensitive period can have profound, often debilitating, consequences. The balance of estrogens and androgens is critical for normal development, growth, and functioning of the reproductive system. Although it is especially important during development, this balance is important throughout life for preservation of normal feminine or masculine traits.

A number of environmental chemicals have actions that mimic or alter the normal sex steroid hormones. The fetus is especially vulnerable because this is the period of time when organs develop. If the normal balance between estrogens and androgens is disrupted, the result may be feminization of males, masculinization of females, birth defects of the reproductive organs, reduced fertility, and alteration of the expression of normal feminine or masculine personality traits, probably including sexual preference. These effects during development are of particular significance because they are often irreversible, and the child must live with the altered reproductive and sexual function for the rest of his or her life. For adults, there is concern about the influence of endocrine-disruptive chemicals on the incidence of cancer of the reproductive organs and on diseases such as endometriosis, fibroids, prostate hyperplasia, and reduced sperm counts.

Much of the evidence for developmental alterations with estrogenic substances comes from the use of diethylstilbestrol by women some years ago as an agent thought to prevent pregnancy loss. This synthetic estrogen caused a series of developmental abnormalities of both male and female genital systems, and it also caused induction of a rare form of vaginal cancer. Newbold (*45*), comparing effects reported in humans and in animals, found immune dysfunction, subfertility, masculinization of females and feminization of males, abnormalities of both the male and female reproductive tracts, sperm abnormalities, and prostatic inflammation upon exposure

to this synthetic estrogen. It is, however, less clear whether environmental estrogens have similar actions, but these abnormalities define the suspected effects of such compounds. Androgen receptors may also be targets of action of xenobiotics, as has been well demonstrated in animal studies (*46,47*). DDE [*p,p′*-bis(4-chlorophenyl)-1,1, dichloroethane], the major metabolite of DDT [bis(4-chlorophenyl)-1,1,1-trichloroethane], is known to be a potent androgen receptor antagonist (*48*).

Although many wildlife and animal studies and a body of information from *in vitro* studies have conclusively demonstrated developmental disruption of estrogenic and androgenic function, this is not so for human studies. The problems of proving that population-based changes in disease patterns and behavior result from exposure to hormonal disruptors is much more difficult for human studies than for animal studies, where one can control exposure to a single contaminant because humans are always exposed to chemical mixtures.

One of the few developmental studies in humans that has convincingly demonstrated effects of specific contaminants is that of the Yucheng cohort in Taiwan, exposed to PCBs and furans from contaminated cooking oil. Male children born to mothers with contamination showed decreased penis length but no effect on testis size or Tanner stages of development (*49*). Blanck et al. (*50*) showed that girls exposed *in utero* to polybrominated biphenyls reached puberty at a younger age than unexposed girls. The findings have some consistency, indicating an increase in the incidence of endometriosis in women exposed to PCBs and/or dioxins (*51,52*), which is consistent with studies in monkeys (*53*).

Studies evaluating possible associations between organochlorine compounds and human male infertility have shown mixed results. The ratio of male to female births was reduced for offspring of men heavily exposed to dioxin in Seveso, Italy (*54*). In a study of 29 patients and 14 controls, significantly higher amounts of tetra- and pentachlorinated biphenyls, DDE, DDT, and lindane (γ-hexachlorocyclohexane) were present in male patients with infertility problems than in the controls (*55*). The levels of three PCB congeners (2,2′,4,4′,5,5′-hexachlorobiphenyl, 2,2′,3′,4,4′,5-hexachlorobiphenyl, and 2,3′,4,4′,5-pentachlorobiphenyl) were inversely correlated with sperm motility index in samples with a sperm count less than 20 million cells/mL (*56*). In contrast, a clinical laboratory investigation of 38 transformer repair workers and 56 control workers with serum PCB levels of 12 ppb and 6 ppb, respectively, did not report differences in sperm count (*57*).

Effects on male reproduction are not limited to organic contaminants. Lead also functions as an endocrine disruptor, causing reduced sperm count, reduced semen volume, and changes in sperm motility and morphology [for a review, see Apostoli et al. (*58*)]. Male lead exposure has been associated with increased infertility (*59*). In a classic study, Lancranjan et al. (*60*) classified 150 occupationally exposed men into four groups: those poisoned with lead, those with moderate levels, those with levels slightly above baseline, and those with physiologic levels. Sperm motility decreased and abnormal sperm morphology increased with increasing lead exposure in a dose–response relation. A recent study of 149 healthy male industrial workers (*61*) reported a significant correlation between blood lead levels and reproductive parameters, including a decrease in sperm density, total counts, motility, and viability. Subpopulations of men with genetic polymorphisms may be at a higher risk for developing fertility problems because of environmental exposures. Benoff et al. (*62*) suggest that Ca^{2+} and K^{+} channel isoforms identified in human testes and spermatozoa may lead to greater sperm damage in men exposed to lead and cadmium.

We have considerable evidence that men in developed countries may have significant decrements in semen quality and quantity, decreased sperm count, and increases in testicular cancer, hypospadias, cryptorchidism, and male breast cancer [for a review, see Toppari et al. (*63*)]. A number of investigators have suggested that these effects are secondary to increased exposure to estrogenic xenobiotics (*64,65*). Whether or not this is the case awaits further study.

Human Diseases Based on Current Understanding of Cellular and Molecular Mechanisms

Neurodegenerative Diseases

The many distinct neurodegenerative diseases share a number of common features. In each, specific populations of neurons die and disappear: upper and lower motor neurons in amyotrophic lateral sclerosis (ALS), neurons in the substantia nigra in Parkinson's disease, and neurons in frontal cortex and hippocampus in Alzheimer's disease. For each, a small minority of cases appear to be genetic, but the vast majority are random, and for each, several different environmental agents are possible contributors to the disease.

There have long been suggestions for a role of metals, including aluminum, iron, and lead, in all three of these diseases, although for none of these diseases are metals likely to be the sole or even primary factors. Aluminum in dialysis fluid causes a clear dementia that appears to be similar to but distinct from Alzheimer's disease (*66*). We have some epidemiologic evidence for the incidence of Alzheimer's disease paralleling that of aluminum in drinking water (*67*). These observations have led to considerable speculation about a role for aluminum in Alzheimer's disease [see, e.g., Yokel (*68*)]. The demonstration that aluminum induces a hyperphosphorylation of tau protein (*69*), a major protein in neurofibrillary tangles, has provided a mechanistic basis for the association. Excess aluminum has been implicated in the high incidence of ALS in the Western Pacific (*70*) and has been proposed as an agent behind the Guam syndrome of dementia, Parkinson's disease, and ALS (*71*).

For many years, evidence has suggested that lead is a risk factor for some neurodegenerative diseases, especially for ALS. Campbell et al. (*72*) and Conradi et al. (*73*) reexamined the hypothesis and reported elevated lead levels in blood and cerebral fluid from ALS patients. Although others have not replicated these observations, they nevertheless acknowledge that lead may be a contributing factor in the pathogenesis of disease (*74–76*). Recent evidence showing that occupations with exposure to welding, soldering, and electric plating increase risk of ALS by 5- to 8-fold is consistent with this suggestion (*77*), and the most likely factor is proposed to be exposure to lead. Prince (*78*) has summarized epidemiologic studies of Alzheimer's disease incidence, concluding that this disease is more common in urban than in rural areas and in developed than in developing countries. He concludes that individuals are at greater risk of Alzheimer's disease if they show subtle neuropsychologic deficits and educational disadvantages and hypothesizes that early lead exposure could explain these findings. These observations are compatible with a number of cellular studies showing that lead greatly amplifies several other forms of neuronal damage [for discussion, see Savolainen et al. (*79*)]. Savolainen et al. (*79*) attribute these actions to lead amplification of neurotransmitter-induced ROS generation, plus an effect of lead to reduce cellular glutathione levels. Iron has been suggested to be a factor in neurodegenerative diseases, especially in Parkinson's disease, on the basis of the observation that levels of iron are elevated in the parkinsonian substantia nigra and that iron induces oxidative stress (*80,81*). Gerlach et al. (*81*) also report elevated iron levels in the caudate, but not the nigra, in the association centers, hippocampus, and basal forebrain in Alzheimer's disease. Lovell et al. (*82*) showed that senile plaques in Alzheimer's

disease patients have elevated levels of iron. Hirsch et al. (*83*) reported elevated levels of both iron and aluminum in substantia nigra from Parkinson's disease patients.

Although the role of organochlorine compounds in neurodegenerative diseases has been less studied, increasing evidence suggests that organochlorines are involved, especially in Parkinson's disease. Schulte et al. (*84*) report that incidences of Parkinson's disease, Alzheimer's disease, and ALS have increased in occupations involving exposure to pesticides and solvents. Specifically for Parkinson's disease, risk factors include rural living, well-water consumption, pesticide exposure, and exposure to solvents (*85,86*). PCBs are elevated in brains of Parkinson's disease patients (*87*), as are dieldrin (*88*) and lindane (*89*). Exposure to paraquat has been reported to cause a 3.6-fold increase in the risk of developing Parkinson's disease (*90*). Bhatt et al. (*91*) report five persons who developed reversible Parkinson's disease symptoms following organophosphate pesticide intoxication. Ferraz et al. (*92*) reported Parkinson's diseaselike symptoms in two farm workers exposed to the fungicide maneb and suggested that the manganese in this fungicide is the major toxic agent.

Several characteristics of dopaminergic neurons of the substantia nigra distinguish them from other neurons, and even from other dopaminergic neurons. These considerations have led to a general belief that excessive generation of ROS is likely to be a major factor in development of Parkinson's disease (*80,93*). Tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, acts by production of a transient substrate–radical–$Fe^{2+/3+}$ complex and thus both uses and produces ROS (*94*). Dopamine is metabolized by monoamine oxidase, and this process results in production of ROS [see McGeer et al. (*95*)]. Dopamine can be oxidized to form superoxide anion radical and reactive quinones; the latter ultimately form neuromelanin, a polymer of oxidized dopamine, which accumulates in these neurons and may cause cell damage (*96,97*). The substantia nigra has much more iron than other brain regions, and through the Fenton reaction, Fe^{2+} can be oxidized to Fe^{3+} and ROS (*98*). Iron binds to neuromelanin (*99*), where it may do double damage (*100*). The excessive production of ROS results in depletion of glutathione and other antioxidants (*101*) and increased lipid peroxidation (*102*).

As for many other diseases, the interactions of the various metals and organics implicated in neurodegenerative diseases have been little studied. Unfortunately, we have few adequate animal models of these diseases, which makes determining how these contaminants interact in these diseases even more difficult.

Cancer

Cancer, in general, results from interactions between environmental exposures and genetics. Genetic factors alone may account for no more than 5% of cancers (*103*). Thus, as for the diseases discussed above, susceptibility is critical to an understanding of cancer, and many environmental agents act to alter susceptibility. Cigarette smoking is the single most significant factor and is responsible for an estimated 30% of all cancer deaths and 85% of lung cancer deaths (*104*). Other factors include diet, pollutants, radiation, infectious agents, and drugs. Despite the fact that genetics alone does not account for most cancers, cancer is essentially a genetic disease, in that environmental agents or viruses can alter the genes regulating cell division. But, the reality is that most of us are not born with a genetic makeup that guarantees that we will develop cancer. Some individuals are at increased risk of cancer because of inherited genetic differences that influence metabolic activation or detoxication of carcinogenic substances, and propensity to these inheritances may depend on ethnicity or race. Age and gender also impart differences in susceptibility, whereas immune suppression or inadequate nutrition may also increase susceptibility (*105*).

The National Toxicology Program (*106*) lists 65 substances that are known human carcinogens, plus over 200 substances reasonably anticipated to be human carcinogens. A number of these listings are actually of chemical mixtures such as tobacco smoke and alcoholic beverages. Others include metals (arsenic, cadmium, hexavalent chromium, and thorium as known carcinogens and beryllium, lead, nickel, and selenium as probable carcinogens), but the great majority are organic molecules. Some of the same factors implicated in neurodegenerative and cardiovascular diseases almost certainly play roles in cancer, especially ROS production and cellular oxidative stress. Ames et al. (*107*) have emphasized the role that antioxidants present in fruits and vegetables play in preventing cancer. ROS production, and/or reduction of cellular systems to scavenge ROS, appears to be a major factor in cell injury, cell death, and aging at many sites.

Endogenous steroidal estrogens function not only as hormones but also as carcinogens [reviewed by Liehr (*108,109*)]. Estrogens have been proposed to induce carcinogenesis by multiple mechanisms including covalent modification of the estrogen receptor (*110,111*), induction of chromosomal abnormalities (*112,113*), an epigenotoxic mechanism (*114*), conversion of 17β-estradiol to catechol estrogens and redox-active and adduct-forming estrogen quinones (*108,115*), and subsequent ROS-mediated DNA damage (*116,117*).

Breast cancer exemplifies the difficulties in understanding the interactions between endocrine and carcinogenic actions. As with all cancers, the etiology of breast cancer is complex, with multiple risk factors. Lifetime exposure to estrogens is a major risk factor for breast cancer (*118,119*). Elevated serum estrogen levels and increased urinary excretion rates of estrone, estradiol, and estriol have been detected in breast cancer patients compared with controls (*118*). Postmenopausal women living in countries with a higher risk of breast cancer, such as the United States or United Kingdom, have elevated levels of urinary and/or serum estrogens compared with women living in countries with a lower risk of breast cancer, such as Japan, China, and Singapore (*120–123*).

The hypothesis that persistent chlorinated hydrocarbons play a role in the etiology of breast cancer is highly controversial (*124–127*). The first study to suggest a correlation between environmental estrogenic xenobiotics and breast cancer was by Falck et al. (*128*). They measured the levels of various chlorinated hydrocarbons in mammary adipose tissue from women with malignant and nonmalignant breast disease and found elevated levels of several compounds including PCBs, DDT, and DDE in tissue from women with malignant disease. Others have found (*129–131*) or not found (*132–136*) a relationship between exposure and disease. A recent meta-analysis of five previously published reports concluded that there was no significant correlation between exposure to organochlorines and breast cancer risk (*137*), although this study could not rule out existence of genetically vulnerable subpopulations.

Feigelson et al. (*138*) proposed a multigenic model of breast cancer based on individual susceptibility. They suggest that genetic differences among women that result in alterations of biosynthesis and metabolism of endogenous estrogens explain differences in breast cancer incidence. Feigelson et al. (*139*) report that genetic polymorphisms in two genes involved in estrogen metabolism, CYP17 and HSD17B1, are related to breast cancer incidence. Moysich et al. (*140*) found that women with a high PCB body burden and a variant CYP1A1 allele had a higher incidence of breast cancer, whereas there was no significant association between PCB concentration and breast cancer among women who did not have the variant allele (*140*). Millikan et al. (*141*) found a statistically significant relationship between risk of breast cancer and serum PCB levels

in African-American women but not in white women, thus adding further support to the possibility that, although the relationship between PCB levels and breast cancer is weak, it is significant for vulnerable populations. Rundle et al. (*142*) reported similar findings from investigating the relationship between DNA damage in breast tissue due to exposure to PAHs and a genetic deletion in the gene encoding a xenobiotic detoxifying enzyme, glutathione *S*-transferase M1. Although the null glutathione *S*-transferase M1 genotype did not predict breast cancer cases, it did predict PAH–DNA adduct levels in malignant versus benign breast tissue, and PAH–DNA adducts are correlated with breast cancer (*143*).

Diseases Involving Both Physiologic Disruption and Cell Damage

Cardiovascular Disease

Cardiovascular disease is the major cause of morbidity and mortality in developed countries such as the United States. Known risk factors for cardiovascular disease, especially ischemic heart disease, include genetic predisposition, high-fat diets, smoking, and lack of exercise. Cerebrovascular disease ("brain attacks") have etiology and risk factors similar to ischemic heart disease that lead to myocardial infarctions.

Smoking is a major risk factor for cardiovascular disease, and certainly smoking is the ultimate example of exposure to a chemical mixture. Cigarette smoking is responsible for approximately 20% of the 500,000 deaths from cardiovascular disease in the United States each year (*104*), and it also contributes to cerebrovascular disease. We have some evidence suggesting that smoking has synergistic, not additive, effects, with the other two major risk factors for coronary heart disease (hypertension and hypercholesterolemia) (*144*). The mechanisms by which smoking results in atherosclerosis are only partially understood, but the well-documented association is enough to indicate that cardiovascular disease may be secondary to exposure to environmental toxicants. In addition, a relatively underappreciated body of evidence implicates certain metals and organochlorine compounds as contributors to cardiovascular disease.

For metals, we have good evidence for increased risk of ischemic heart disease from arsenic exposure (*145*), and the elevated risk (2.5-fold) remains after adjustment for several other factors including hypertension and diabetes. This effect also appears to be independent of the peripheral artery disease seen in arsenic poisoning. Mercury from fish has been reported to lead to increased risk of myocardial infarction, possibly through promotion of lipid peroxidation (*146*). Sørensen et al. (*147*) have reported that prenatal methyl mercury exposure results in higher blood pressure at seven years of age. Lead also has serious cardiovascular effects, altering electrical activity and promoting atherosclerotic changes and response to transmitters (*148*). These actions are in addition to the evidence that chronic lead poisoning induces hypertension (*149*). The mechanisms responsible for all these actions of metals are unknown.

Studies of workers exposed to phenoxy herbicides and dioxins have reported a significant elevated overall morbidity and an elevated risk of ischemic heart disease (*150,151*). In the study of Flesch-Janys et al. (*150*), the risk for morbidity from ischemic heart disease increased in a dose-dependent fashion with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and with all polychlorinated dioxin and furan exposures combined. For the highest exposure category, the risk ratio was 2.48 (95% confidence limits, 1.32–4.66). Michalek et al. (*152*) have reported a significantly elevated increased risk of death from circulatory system diseases among enlisted ground troops exposed to dioxin in Agent Orange in Vietnam. Vena et al. (*153*) reported an increased risk for circulatory diseases, especially ischemic heart disease, in dioxin-exposed workers for an International Agency for Research on Cancer international cohort (relative risk, 1.67; 95% confidence limits, 1.23–2.26). Cardiovascular disease has been found to be elevated in the Seveso population exposed to dioxin after a herbicide plant explosion (*154*). Because coplanar PCBs are dioxinlike, these observations are relevant to PCB exposure as well. Some studies show that PCBs contribute more to toxic equivalents for dioxin than TCDD itself (*155*).

The mechanisms whereby dioxinlike substances alter cardiovascular function are uncertain, but the evidence suggests that both physiologic changes and direct cell damage are involved. Serum lipids are elevated in PCB-exposed (*156–159*) and dioxin-exposed (*160*) populations. Animal studies have shown that sublethal exposure to TCDD is associated with effects on lipid metabolism (as reflected by increased serum cholesterol, as well as generally increased serum triglyceride concentrations) (*161*). Monkeys exposed to dioxin and PCBs showed a 3- to 5-fold elevation in serum triglyceride concentrations (*53*) similar to what has been found in numerous other animal studies [see Safe (*162*)]. The abnormal lipid levels reflect altered liver function. The liver increases in size after exposure to TCDD in animals (*163*) and humans (*164*), and the increased serum lipids reflect induction of lipogenic enzymes (*165*). Elevated serum lipids are a well-documented risk factor for atherosclerosis and ischemic heart disease. Furthermore, animal studies have shown that dioxin and coplanar PCBs cause the production of ROS (*166,167*), which in turn causes damage to endothelial cells and promotes the formation of foam cells and atherosclerotic plaques (*168–170*). These actions are similar to those caused by PAHs in tobacco smoke in human endothelial cells (*171*). The combination of elevations of serum lipids in the presence of damage to endothelial cells is a clear formula for cardiovascular disease.

Cerebrovascular disease has essentially the same etiology as cardiovascular disease. However, we are unaware of any studies in the past that have investigated a relationship between cerebrovascular disease and xenobiotic exposure, but we would expect the risk factors to be similar to those for ischemic heart disease.

Two important considerations relate to cardiovascular disease. Despite the well-known relationship to smoking, cardiovascular disease is not widely recognized as being related to environmental factors; nevertheless, we have increasing evidence that a variety of environmental agents contribute to this disease. Furthermore, in addition to the direct damage that these toxins cause on endothelial cells, alteration of normal liver function, as reflected in abnormalities of lipid metabolism, can serve as a factor that significantly increases risk of development of cardiovascular and cerebrovascular diseases.

Cellular and Animal Models of Environmentally Induced Disease

Steroidal Hormone Function and Chemical Mixtures

Steroid hormones are lipid molecules with limited solubility in plasma and are accordingly carried through the plasma compartment to target cells by specific plasma transport proteins. Each transport protein has a specific ligand-binding domain for its associated hormone. It is generally accepted that the "free" form of the steroid hormone, and not the conjugate of the hormone with its plasma transport protein, enters target cells and binds with the appropriate receptor. Receptors for the steroid hormones are proteins located primarily in the cell nucleus or partitioned between the cytoplasm and the nucleus. The unoccupied steroid receptors may reside in the cell as heterodimeric complexes with the 90-kDa heat-shock protein, which prevents the receptor from binding with the DNA until the receptor has first bound with its steroid hormone. Once the

hormone binds to the receptor, the hormone receptor complexed with the HR-HSP undergoes a conformational change and is considered to be activated. The activated receptor binds with DNA at a specific site, initiating gene transcription and eventually resulting in a specific biologic response (e.g., cell proliferation and tissue restructuring). The regulation of steroidal endocrine systems occurs through a series of feedback mechanisms involving both hormone production and receptor levels in target tissues.

Theoretically, environmental chemicals that disrupt normal hormone function could interfere at any of the steps described above. However, most recent investigations have focused on environmental chemicals referred to as hormonally active agents, or xeno-estrogens and xeno-antiestrogens, which act through binding to the estrogen receptor (ER). A chemical mixture may contain a number of xeno-estrogens that agonistically bind the ER, enhancing the response of endogenous estrogens, or it may contain a number of xeno-antiestrogens that antagonistically bind the ER, inhibiting the normal action of endogenous estrogens. Some estrogenic compounds in chemical mixtures may exert an overall estrogenic response not by binding to the ER but rather by binding to estrogen plasma transport proteins, resulting in more "free" endogenous estrogen. A mixture containing both xeno-estrogens and xeno-antiestrogens may have no net biologic response in the organism.

Many environmental chemicals have no measurable estrogenic/antiestrogenic activity in simple *in vitro* systems yet produce significant activity *in vivo*. This is the case with numerous compounds that are activated by any one of the P450 cytochromes (P450 or CYP). P450s are the stalwarts of our detoxification system and, as such, are involved in the metabolism of most xenobiotics as well as the metabolism of steroid hormones. Xenobiotics and steroid hormones also induce P450s. Environmental mixtures may contain chemicals that induce P450s, are metabolized by P450s, or both. Induction of CYP1A1 and/or CYP1B1 occurs when xenobiotics such as TCDD or PAHs bind the AhR, and may result in antiestrogenic activity through increased metabolism and depletion of endogenous estrogens (*172*). On the other hand, compounds that are metabolized by P450s may result in a net estrogenic effect if they inhibit endogenous estrogens from being metabolized. Figure 1 depicts some of the ways in which an environmental toxicant may disrupt normal steroid function. Clearly, predicting the activity of a chemical mixture containing a combination of xenoestrogens/antiestrogens and P450 inducers and substrates on hormonal function is a humbling task.

Research efforts in a number of laboratories [for reviews, see Liehr (*173*) and Parl (*174*)] have led to the development of a conceptual model of breast cancer that includes the carcinogenic and hormonal activity of endogenous estrogens, takes into consideration genetically high-risk populations, and can be expanded to include the role of chemical mixtures in the development of breast cancer. In the first step of this model, an environmental chemical or estradiol is converted in mammary tissue to a genotoxic agent (an activated PAH or catechol estrogen–derived quinone, respectively). In the second step, the enzyme(s) involved in the activation of the environmental chemical or the metabolism of estradiol exists as a wild-type and genetic variant differing in activity level and results in a higher level of genotoxicity in subpopulations. In the third step, estradiol increases proliferation of ER-positive cells. Thus, in the presence of estradiol, if both an ER-negative and an ER-positive cell have randomly undergone a malignant transformation, the ER-positive cell will be selected, whereas the ER-negative cell will grow less quickly, allowing time for DNA repair or apoptosis. This model explains why more than 60% of all breast tumors are ER positive, whereas fewer than 20% of normal breast epithelial cells are ER positive.

Although numerous chemicals induce mammary tumors in laboratory animals, no chemical carcinogen of the human breast has been unequivocally identified. Although individual pesticides, PCBs, and hydroxylated PCBs are estrogenic *in vitro* and *in vivo*, the estrogenic activity is apparent at high (micromolar) concentrations only (*14,175–179*). However, Sarkar et al. (*180*) suggest that further investigation of ethanol-associated damage to breast cell DNA resulting from ROS is warranted. Recently, data have been presented suggesting that alcohol consumption may result in increased bioactivation of the mutagen/carcinogen acetaldehyde and/or free radicals. Castro et al. (*181*) demonstrated that incubation of cytosol prepared from rat mammary tissue with a xanthine oxidoreductase cosubstrate (nicotinamide adenine dinucleotide, hypoxanthine, xanthine, caffeine, theobromine, or 1,7-dimethylxanthine) significantly enhanced the biotransformation of ethanol to acetaldehyde. These data suggest that the *in situ* bioactivation of ethanol to a carcinogen such as acetaldehyde and potentially to free radicals may be involved in alcohol-induced breast cancer. The consumption of foods that provide cosubstrates for xanthine oxidoreductase, such as caffeinated beverages and purine-rich meats, may enhance the biotransformation of ethanol to acetaldehyde.

PAHs, a group of environmental pollutants, mutagens, and carcinogens, are bioactivated primarily by P450s and epoxide hydrolase. The resulting reactive PAH-diolepoxides can bind DNA, forming PAH–DNA adducts. Higher levels of PAH–DNA adducts have been detected in the breast tissue of cancer patients compared with that of controls (*143*). In addition to binding DNA, certain PAHs and/or their metabolites bind the ER (*182–186*). It has been suggested that by binding ERs, PAHs may accumulate in the nucleus of cells, resulting in increased rates of mutagenesis (*183*). Although this hypothesis has not been adequately tested, it

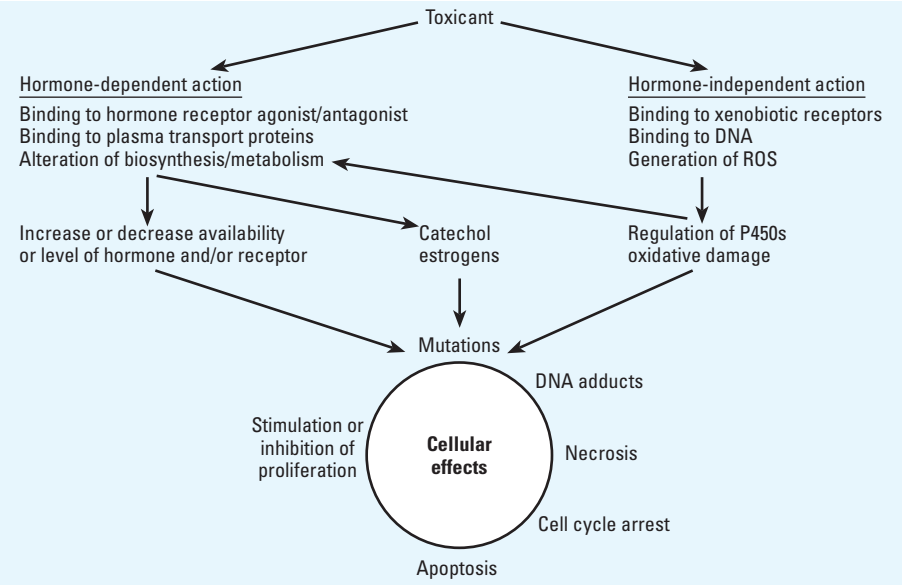

Figure 1. Toxicants as modulators of steroid hormone function.

is supported by the finding that high doses of estrogens or antiestrogens, given simultaneously or prior to a dose of the mutagenic PAHs 3-methylcholanthrene or 7,12-dimethylbenz[*a*]anthracene, inhibit the development and/or growth of mammary tumors (*187–189*). Presumably, the estrogens and antiestrogens occupy the ER, preventing the binding of the PAH to ERs, inhibiting mutagenesis. Humans are continuously exposed to and have detectable levels of PAHs, thus making these compounds potentially important genotoxic agents in the initiation of breast cancer.

Results from numerous studies indicate that many chemicals in the environment, including pesticides, PCBs, PAHs, phthalates, phytoestrogens, and others, are estrogenic in *in vitro* assays. However, the potency of these compounds is very low compared with endogenous estrogens (10,000- to 200,000-fold lower), and it is uncertain whether humans ever experience an environmental mixture that results in a net estrogenic effect. For example, individual pesticides, PCBs, and hydroxylated PCBs are estrogenic *in vitro* and *in vivo* in animals but only when present at micromolar concentrations (*14,175–179*). Rubin et al. (*190*) reported that perinatal exposure to low doses of bisphenol A in drinking water affects body weight, estrus cycle patterns, and plasma luteinizing hormone levels in adult rats, and that lower doses had a greater effect than higher doses. The concentrations used were below those giving a uterotropic response in ovariectomized females, indicating a unique susceptibility in the developing organisms. However, in general these compounds are in human blood and tissue at nanomolar, not micromolar, concentrations. Furthermore, it is not clear whether the estrogenic activities of multiple individual compounds are additive in human tissue. Nevertheless, the fact that some of these xenobiotics are persistent may result in effects despite their low potency.

Two recent papers suggest that the total activities of a mixture of individually weakly estrogenic organochlorines are additive, and that weakly estrogenic compounds can produce additive effects with low levels of steroidal estrogens. Using two standard pharmacologic methods for modeling the interaction of chemicals in mixtures (concentration addition and independent action models), Payne et al. (*191*) determined that the combined effect of *o,p′*-DDT, *p,p′*-DDE, β-hexachlorocyclohexane, and *p,p′*-DDT on proliferation of MCF-7 cells was additive even when the individual compounds were present at levels below their individual no-observed-effect concentrations. Rajapakse et al. (*192*) used a yeast reporter gene assay for bisphenol A and *o,p′*-DDT to investigate the combined action of weakly estrogenic chemicals with estradiol. They found that "at approximately equieffective concentrations corresponding to molar mixture ratios between 1:20,000 and 1:100,000 (estradiol:BPA or *o,p′*-DDT) substantial modulations of the effects of estradiol became discernible." These data suggest that mixtures of weakly estrogenic compounds are most likely to contribute to an estrogenic effect in humans when the estradiol levels are very low and the xenoestrogen concentrations are highest, a condition most likely to occur in children. Rajapakse et al. conclude that the potential health implications of additive combination effects between xenoestrogens and steroidal estrogens deserves serious consideration.

Whether a mixture of weakly estrogenic chemicals results in estrogenic activity depends not only on the concentration but also on the combination of individual compounds. Hany et al. (*193*) reported that a mixture of PCBs prepared to reflect the PCB congeners and concentrations routinely detected in human breast milk was estrogenic. Female rats whose mothers received the PCB mixture had increased uterine weights at 21 days old. Other PCB mixtures tested at similar concentration were not estrogenic.

Du et al. (*194*) measured doubling times in MCF-7 cell cultures treated with either estradiol or one of two Chinese PCB mixtures, PCB3 and PCB5, which are similar in congener composition to U.S. Aroclors 1242 and 1254, respectively. Relative proliferation effects were calculated on the basis of decreases in doubling time compared with vehicle control and estradiol. PCB3 and PCB5 were as potent as estradiol, producing 100% of estradiol's proliferative effect at concentrations of 0.03 and 0.6 nM. At 30 nM, treatment with PCB3 still resulted in an 86% proliferative effect, whereas PCB5 showed no proliferative effect. Du et al. suggest that PCB5 may have been toxic at 30 nM. These results are startling because all previous reports have suggested that individual PCB congeners and mixtures are estrogenic in the micromolar range and show no effect at nanomolar and picomolar levels. The reason for this discrepancy is unknown, but it may be that PCB3 and PCB5 contained a potent estrogenic contaminant other than the PCB congeners.

Most of the mixtures to which humans are exposed contain a variety of compounds with estrogenic and antiestrogenic effects. Results from a number of studies suggest that metabolism of environmental chemicals may produce more potent estrogens/antiestrogens (*186,195*). Also, most assays measure the estrogenic activity of a compound on basis of binding to ER and activation of transcription. As mentioned previously, a chemical agent can produce an estrogenic response *in vivo* by other mechanisms (e.g., alteration of ER levels, alteration of endogenous estrogen metabolism). Therefore, it is plausible that chemical mixtures may be more estrogenic/antiestrogenic *in vivo* than predicted based on results from *in vitro* assays. Another indication that mixtures of environmental compounds may be antiestrogenic is the finding from a comprehensive chronic toxicity study of four commercial PCB mixtures (Aroclors 1016, 1242, 1254, and 1260) in which rats received one of the mixtures for 24 months. Females fed the mixtures with the highest chlorine content (Aroclor 1260) had a significantly decreased incidence of mammary neoplasms compared with the control group (*196*).

Reduced Male Fertility

Perinatal exposure to chemical mixtures alters sexual differentiation and may disrupt normal hormone function if the chemicals act as antiandrogens, estrogens, AhR aromatic hydrocarbon receptor agonists, or fetal germ cell toxicants. Antiandrogens bind the androgen receptor without activating it. Antiandrogenic activity has been noted for a number of different types of chemicals, including pharmaceuticals (hydroxyflutamide), pesticides (procymidone, vinclozolin, *o,p′*-DDE), fungicides (vinclozolin), and estrogens (diethylstilbestrol and estradiol). Perinatal exposure to antiandrogens can be detected in laboratory animals as decrease in anogenital distance, nipple retention, hypospadias, delay in preputial separation, decrease in sex accessory gland weights, and inhibition of endogenous gene expression. Estrogens can produce antiandrogenic effects either by inhibition of testicular androgen secretion via blocking the secretion of luteinizing hormone or by direct suppression of testosterone synthesis by Leydig cells. Estrogens also alter male reproduction through binding with the ER and activation of specific gene responses. The antiandrogenic effects of estrogens are detectable *in vivo* in male laboratory animals as alterations in mating behavior, serum levels of luteinizing hormone, and spermatogenesis. AhR agonists (TCDD, non-*ortho*-substituted PCBs) bind the AhR and induce P450s that can activate procarcinogens and alter the metabolism of steroid hormones.

The environmental toxicant whose adverse effects on male reproduction have been the most studied is TCDD. In a study by Moore et al. (*197*), adult male rats given single doses of TCDD exhibited decreases in plasma testosterone and dihydrotestosterone concentrations by 90 and 75%, respectively,

and decreased seminal vesicle and ventral prostate weights. Moore et al. concluded that whereas the TCDD-induced depression in plasma testosterone concentration appeared to be the primary event observed, the mechanism by which testosterone concentrations decreased remains unknown.

A series of studies conducted to examine the effects of *in utero* and lactational exposure to TCDD on adult male rats showed a decrease in androgenic status (lowered testosterone levels), altered sexual behavior, decreased serum luteinizing hormone levels, and decrements in spermatogenesis and reproductive capability (*198–200*). Another series of studies reported different effects for *in utero* versus lactational exposure (*201*), a decrease in the responsiveness of ventral prostate after *in utero* and lactational exposure (*202*), and partial demasculinization and feminization of male sex behavior with *in utero* and lactational exposure (*203*). Gray et al. (*204*) showed that prenatal exposure to TCDD permanently reduced sperm number and sex accessory weights but did not alter testosterone levels. Furthermore, a single exposure to TCDD on gestational day 15 was sufficient to permanently reduce epididymal sperm reserves (*205*). It has often been stated that the effects of TCDD are at least partially due to catabolism of testosterone because TCDD is a potent inducer of P450s. Although it is clear that TCDD can increase the catabolism of testosterone *in vitro*, a study that measured TCDD-induced catabolism *in vivo* found no evidence that TCDD altered the disappearance rate of testosterone (*206*). At this time how TCDD produces its multiple effects on male reproduction is still not clear.

Reproductive effects of lead exposure have been well documented in laboratory animals. In general, results from the experimental studies are consistent with the epidemiologic studies. Exposure to lead acetate in drinking water results in a decrease in sperm count (*207–209*), a decrease in basal and chorionic gonadotropin-stimulated testosterone production (*210*), and a decrease in serum testosterone as well as early onset of capacitation (*211*).

Numerous mechanisms of action have been demonstrated or suggested to account for the observed effects of PCBs and inorganic lead on the male reproductive system. It is clear that perinatal exposure to either Aroclor 1242 or 1254 results in hypothyroidism (*212*). Because thyroid hormones directly suppress mitogenesis of neonatal Sertoli cells, the hypothyroidism may induce increased neonatal Sertoli cell proliferation, and a larger Sertoli cell population may be responsible for the increases in germ cell numbers, testis weight, and daily sperm production that characterize this phenomenon. Support for this hypothesis comes from the results of a Sertoli cell proliferation assay in PCB-treated animals (*212*). Fifteen-day-old rats (control and PCB treated) received tritiated thymidine via injection 2 hr before death, and their testes were fixed in 10% formalin. Analysis of incorporation of [^{3}H]thymidine revealed greater proliferation in Sertoli cells in testes from PCB-treated animals than in testes from controls. Although the increase in testis weight induced by PCBs was less than that induced by the goitrogen 6-propyl-2-thiouracil, both PCBs and 6-propyl-2-thiouracil effectively reduced the thyroid hormone thyroxine (T_4). Thus, it appears that factors other than the reduction of T_4 are affecting the testes weights of PCB-treated male rats.

PCBs bind the AhR and induce P450s that may catalyze the catabolism of steroid hormones or decrease the production of testosterone. Machala et al. (*213*) studied the effects of chronic exposure to PCBs on cytochrome P450 systems and steroidogenesis in liver and testes of the bull and observed inductions of hepatic ethoxyresorufin *O*-deethylation and 6β-hydroxylation of testosterone. They also found a high level of PCB-inducible androstenedione formation. PCB exposure reduced testicular microsomal P450s and affected androstenedione formation and 16β-hydroxylation of testosterone. Mitochondrial CYP11A, the rate-limiting enzyme of steroidogenesis, was inhibited by 50% in the testes of exposed animals. Machala et al. suggest that this activity, as well as the hepatic testosterone 6β-hydroxylation and hepatic and testicular androstenedione formation, may significantly contribute to the decrease in testosterone levels often observed with PCB exposure. In the above-described study and others, it appears that perinatal exposure to PCBs can result in "enzymatic imprinting." In another study, a single perinatal exposure to Aroclor 1254 permanently altered the adult expression of hepatic and testicular P450s (*214*).

Oxidative Stress and DNA Damage in Sperm

Phospholipids are the major component (60–70%) of the lipids in sperm cells (*215*), and the phospholipids have a high content of polyunsaturated fatty acids. Consequently, sperm cells are susceptible to lipid peroxidation from various ROS such as superoxide anions, hydroxyl radicals, and hydrogen peroxide. Jones et al. (*216*) found that more than 60% of the major polyunsaturated phospholipid fatty acid in human spermatozoa, docosahexaenoic acid, was lost after exposure of the spermatozoa for 2 hr to the prooxidant catalyst system of iron/ascorbate. The fatty acid degradation was accompanied by an increase in the lipid oxidation byproduct malondialdehyde (MDA). This degradation of phospholipids can lead to destruction of cell membranes. Furthermore, the ROS can react with DNA molecules, leading ultimately to mutations affecting both spermatogenesis and the genetic status of progeny. Investigations into the causes of infertility in human male populations have shown that MDA levels and/or levels of a biomarker for oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG), were statistically associated with decreases in indices of sperm quality such as sperm number and sperm motility (*217,218*). Oxidation of spermatozoa *in vitro* also demonstrated that 8-OHdG levels were negatively correlated with sperm motility. However, one study showed that the MDA levels in seminal plasma from males who were infertile and had poor indices for sperm quality were similar to MDA levels in seminal plasma from fertile males with good indices of sperm quality (*219*). These results can be explained by the data obtained by Jones et al. (*216*), who found that lipid peroxidation took place within the sperm cells, whereas the seminal plasma had antioxidant properties.

When infertile men received the antioxidants vitamin E and/or vitamin C, their *in vitro* fertilization rates increased and sperm MDA levels decreased (*220*), and sperm concentration increased and sperm 8-OHdG decreased (*217*). Depletion of dietary vitamin C had the effect of increasing sperm 8-OHdG levels (*221*). Cigarette smoking is a life-style factor that has adverse effects on sperm, and it also leads to increases in sperm 8-OHdG (*222,223*). Alcohol consumption has been associated with sperm damage, and we have evidence that alcohol consumption leads to increased oxidative stress in many tissues, including the formation of 8-OHdG in hepatic mitochondrial DNA of rats (*224*). However, an association between alcohol consumption and oxidative damage in sperm has not been reported in the literature.

Exposure to lead increases generation of ROS. Using an assay based on the chemiluminescent signal produced from the reaction of luminol with ROS, Hsu et al. (*225*) found that sperm from lead-exposed rats had increased ROS levels that were inversely correlated with sperm count, motility, and oocyte penetration rate. The mechanism of the formation of ROS by lead has not been resolved. Marchlewicz et al. (*226*) demonstrated that many spermatozoa of lead-exposed rats had abnormal reactions of antioxidant enzymes in the midpiece, suggesting that impairment of free-radical

scavenging enzymes may be an important factor in lead-induced ROS formation. Although no studies report oxidative stress in sperm following exposure to PCBs, the *ortho*- and *para*-hydroxylated metabolite of PCBs can be converted to quinones by peroxidases (*227*). The quinones can undergo redox cycling to produce ROS, and 8-OHdG is formed in an *in vitro* system of calf thymus DNA, lactoperoxidase, and 3,4-dichloro-2′,5′-dichlorobiphenyl (*228*). Therefore, a high potential exists for oxidative reactions to occur in sperm cells exposed to PCBs and other organochlorines because compounds in these cells are readily oxidized by a variety of chemicals as described above.

Molecular Mechanisms of Toxicant Interactions

Deciphering and predicting the complex interactions among organic, inorganic, and organometallic toxicants at the cellular and molecular levels present enormous challenges to toxicologists. A number of recent advances in the characterization of responses to xenochemicals at the molecular level have advanced our understanding of potential interactions among toxicants. Receptors may act as chemical sensors that mediate responses to stimuli by activating stress–response systems, inducing detoxification enzymes, and mounting cellular responses to hypoxia and oxidative stress. Additional signals may be initiated or blocked by the binding of toxicants or metabolites to hormone receptors and transporters. In the past several years research has further elucidated molecular pathways of signal transduction and cross-talk among the various stress–response pathways. Moreover, recent identification of the potential roles of proteins previously referred to as "orphan receptors" in mediating responses to mixtures of xenochemicals has greatly advanced our understanding of the molecular events subsequent to toxicant exposure. Ongoing identification and characterization of the human homologs of receptors and signaling pathways that have been described in experimental animals will help us to determine the human responses to xenochemicals and mixtures thereof and to identify and explain differences in susceptibility between humans and other species.

Chemical Mixtures Activate Multiple Receptors

Activation of gene transcription by mixtures of xenochemicals may involve multiple receptors and signaling pathways. The role of multiple receptors in endocrine disruption has long been hypothesized to be the result of the induction of various phase I and phase II enzymes that catalyze the metabolism of xenobiotics and endogenous hormones (*229*). The inductive responses to xenobiotics have long been referred to as the 3-methylcholanthrene (3-MC) type, phenobarbital (PB) type, or mixed (*230*) because various compounds and mixtures elicit patterns of gene expression similar to the PAH 3-MC, the barbiturate PB, or a mixture of the two. The hallmark of the PB-type response is the induction of P450s of the CYP2B subfamily, whereas the 3-MC–type response is indicated by induction of P450s of the CYP1A subfamily. In addition to 3-MC and PB, the steroid derivative pregnenalone 16α-carbonitrile (PCN) is a known inducer of gene expression, notably of the genes encoding P450s of the CYP3A and CYP2B subfamilies; a number of xenochemicals also appear to mimic PCN in inducing gene transcription. Probably the best example of the activation of multiple receptor pathways after exposure to a chemical mixture is that elicited by Aroclor formulations of PCBs because they comprise complex mixtures of various di-*ortho*-, mono-*ortho*-, and non-*ortho*-substituted congeners with differing degrees of chlorine substitution. Aroclors elicit 3-MC–, PB-, and PCN-type responses in rodents (*231,232*), and PCB-exposed humans appear to show a similar mixed pattern of enzyme induction (*233*).

It is well established that the 3-MC type of enzyme induction profile elicited by chlorinated dioxins and dibenzofurans, PAHs, and non-*ortho*-substituted PCBs is mediated by the AhR. Both the AhR and its heterodimerization partners, the AhR nuclear translocator (ARNT) and ARNT2 (*234,235*), are members of the Per-ARNT-SIM (PAS) family of basic helix-loop-helix transcription factors, which also includes hypoxia-inducible factor 1α (HIF-1α). In contrast, current efforts have shown that the PB- and PCN-type responses are mediated by members of the nuclear receptor family of transcription factors that includes the steroid and thyroid hormone receptors. Recent studies have provided convincing evidence that two orphan receptors of the nuclear receptor family, the constitutive androstane receptor (CAR; also referred to as the constitutively active receptor) and the pregnane X receptor (PXR), mediate the PB- and PCN-type responses (*236–238*).

CAR and PXR as Mediators of the Mixed PB- and PCN-Type Responses

Changes in gene expression in response to exposure to a variety of nonplanar xenochemicals, including *ortho*-substituted PCBs and various pesticides, were similar to those elicited by PB and PCN. Because mixtures may contain multiple agonists/antagonists of several receptors and because the genes that are induced by PB and the genes induced by PCN overlap (e.g., both chemicals induce P450s of the CYP2B and CYP3A subfamilies), the factors controlling gene transcription have been unknown until quite recently. Identification of the PB-responsive elements in the 5′ regions of the CYP2B genes (*239*) and subsequent identification of CAR as the receptor that is activated by PB exposure were major advances (*240–242*). Similarly, genetic elements responsible for the binding of PXR have been identified (*238*). Both PXR and CAR form heterodimers with the retinoid X receptor (RXR) to activate gene transcription, and recent studies indicate that the two receptors can bind the same DNA elements (*243*), which explains the ability of PB to cause some induction of CYP3A and PCN to induce CYP2B. Although the effects of various xenochemicals on PXR and CAR are under investigation, we now know that that *ortho*-substituted PCBs activate the mouse PXR (*244*) and human CAR (*240*). The identification and characterization of RXR, PXR, and CAR from rodents and humans, and studies of their interactions with the regulatory elements of a number of genes have indicated that ligand affinity and specificity cannot be assumed to hold in across-species comparisons (*245*); agonist/antagonist interactions with the human receptors must be evaluated. The recently developed cellular and molecular techniques and assay systems using reporter genes will allow us to determine binding affinity and efficacy of agonists and antagonists of these receptors *in vitro*. The genes encoding xenobiotic-metabolizing enzymes, P450s, and glucuronosyltransferases are the most thoroughly characterized regarding induction by PB and PCN, implicating the involvement of CAR and PXR. It is thought that the expression of a significant number of other genes is regulated by the action of CAR and PXR (*246*) and that regulation by CAR and PXR is not limited to the liver but is observed in extrahepatic tissues including brain (*247*).

Mixtures and AhR-Mediated Effects

Many environmental mixtures contain numerous AhR agonists, principally PAHs, PCDDs, polychlorinated dibenzofurans, and PCBs. Exposure to AhR agonists may occur as a result of various combustion sources, including open burning (*248*) and environmental tobacco smoke. Although individual compounds and mixtures will elicit varied responses because of agonist/antagonist properties and persistence of the response dependent on rate of metabolism of the ligands, the mechanism of AhR-mediated gene transcription elicited by an agonist is currently well defined. Upon ligand binding and release of 90-kDa HSP, ARNT is bound

and the AhR–ARNT complex enters the nucleus, where its binding to xenobiotic-responsive elements found in gene 5′-regulatory regions leads to induction of genes encoding several phase I and phase II enzymes referred to as the aromatic hydrocarbon (Ah) gene battery. Enzymes of the Ah gene battery whose expression is directly regulated by the AhR include the P450s, CYP1A1, and CYP1B1, UDP-glucuronosyltransferase 1A6, glutathione *S*-transferase Ya, NAD(P)H:quinone reductase 1 (NQO1), and class 3 aldehyde dehydrogenase. The molecular events leading to transcriptional activation of the archetypal AhR-regulated gene CYP1A1 have been characterized in considerable detail (*249,250*). Recent studies have focused on the detailed functions of specific domains of the AhR (*251*), functions of the AhR within the chromatin setting (*252*), interactions of the AhR with other proteins that either enhance (*253–257*) or inhibit (*258*) its function, and regulation of the AhR response by proteosome-mediated degradation of the AhR (*259,260*). The recent identification and characterization of the gene encoding the AhR repressor (*261*) represent yet another level of regulation in the complex control of AhR-mediated responses. Recent results support the longstanding hypothesis that the AhR is a receptor for some unidentified endogenous hormone or autocrine factor (*262–264*) and continue to spur efforts to identify endogenous AhR ligands. Although a number of endogenous compounds have been shown to activate the AhR, the most likely candidate activators appear to be derivatives of indole (*265*).

Of the diseases involving the AhR and AhR-regulated genes, PAH-induced lung cancer may be one for which we have a significant degree of understanding of the role of toxicants at the molecular level (*266*). The metabolic activation of PAHs to the ultimate carcinogens, the diol epoxides, was elegantly described over 20 years ago (*267,268*). The enzymes primarily responsible for the metabolic activation of PAHs including benzo[*a*]pyrene are CYP1A1, CYP1B1, and, to a lesser extent, CYP1A2 in concert with epoxide hydrolase (*269*). Both CYP1A1 and CYP1B1 are expressed in human lung (*270*). CYP1B1 appears to be more active than CYP1A1 in the conversion of a number of PAHs to genotoxic intermediates (*269*). In the presence of epoxide hydrolase, both CYP1A1 and CYP1B1 catalyze the conversion of the archetypal PAH, benzo[*a*]pyrene, to its 7,8-dihydrodiol, and both enzymes can in turn metabolically activate this benzo[*a*]pyrene metabolite to a mutagenic form (*269,271,272*). Covalent adducts, formed by the reaction of PAH diol epoxide metabolites with guanine in mutational hotspots of critical genes such as that of the p53 tumor suppressor (*273,274*), may initiate tumorigenesis if not efficiently repaired. PAHs are invariably found as mixtures in the environment (e.g., products of partial combustion, coal tars, environmental cigarette smoke), and the interactions among PAHs may be quite complex. The toxicity and carcinogenicity of a PAH mixture may depend on the net activities of CYP inducers (AhR agonists or antagonists), the prevalence of enzyme inhibitors versus substrates for bioactivation, and the activation or inhibition of metabolic pathways leading to nontoxic/non-carcinogenic metabolites (*275–279*). Results of recent studies indicate that the formation of diol epoxides may not be the only metabolic pathway involved in PAH-induced carcinogenesis. Burczynski and Penning (*280*) have recently shown the propensity of various PAH metabolites to be oxidized to carcinogenic quinones catalyzed by aldo-keto reductases. Some of the PAH-derived quinones are also AhR agonists. The pivotal role of NQO1 in preventing cellular damage and mutations caused by PAH-derived quinones (*281*) was shown in studies with the NQO1 gene knockout mouse, which is more susceptible to PAH-induced carcinogenesis (*282,283*).

Although we understand the roles of AhR-regulated enzymes in PAH-induced cancers reasonably well, a number of toxic effects of AhR ligands, including the hallmarks of TCDD toxicity, the wasting syndrome and immune suppression, have been difficult to explain in terms of the induction of the Ah battery of xenobiotic-metabolizing enzymes. Recent studies have shown that the AhR-mediated responses are not limited to effects involving these enzymes; rather, AhR ligands affect development (*284*), regulation of the cell cycle (*262*), and apoptosis and may do so through a subset of molecular interactions that are only currently being defined. The teratogenic effects of TCDD have long been known, and a role of the AhR in development has been suspected. Targeted inactivation of the AhR gene has indicated physiologic roles of the AhR in normal development in the absence of exogenous AhR ligands because AhR gene knockout mice showed developmental abnormalities including liver defects and delayed population of peripheral organs of the immune system (*284*). Interestingly, inactivation of either of the genes of the dimerization partners of the AhR, ARNT or ARNT2, is far more devastating than inactivation of the AhR gene because both ARNT and ARNT2 gene disruptions are embryonically lethal (*235,285*). This has been attributed largely to the fact that ARNT and ARNT2 are also hybridization partners of HIF-1α (ARNT is also referred to as HIF-1β) and possibly other PAS proteins; lethal defects in angiogenesis and placental development of ARNT have been attributed to disruption of HIF-1α signal transduction. Studies employing conditional disruption of the ARNT gene have confirmed that both AhR-mediated and HIF-1α–mediated gene transcription are lost when the ARNT gene is inactivated (*286*).

Recent studies suggest cross-talk between the AhR-mediated and HIF-1α–mediated pathways, which may explain additional effects of TCDD and other AhR agonists (*287–289*). If competition for ARNT exists between AhR and HIF-1α, TCDD and other persistent AhR agonists may, through sequestration of ARNT, compromise HIF-1α–mediated gene transcription. HIF-1α regulates expression of vascular endothelial growth factor, glucose transporters, and glycolytic enzymes. Because deficits in both glucose transport (*290*) and the activities of several glycolytic enzymes have been implicated in the wasting syndrome of acute TCDD toxicity, this proposed mechanism of AhR/HIF-1α cross-talk has received considerable attention. This cross-talk has been suggested to explain some of the effects of TCDD, such as those on vascularization that have been observed in some animal models, that have not been described mechanistically. It would seem to follow from this mechanism that induction of enzymes and xenobiotic or endobiotic metabolism would not be involved in some of the more profound effects of AhR ligands because the ability of compounds and mixtures to cause persistent activation of the AhR would appear to be the critical factor in the sequestration of ARNT.

AhR agonists have also been shown to exert effects is on cell proliferation. Studies from several laboratories indicate that AhR is involved in the regulation of progression through the cell cycle (*262,291–293*) and that the AhR may also influence whether cells undergo apoptosis (*294*). The AhR, but not ARNT, was recently shown to interact directly with the retinoblastoma protein (RB), delaying cell cycle progression (*291,292*). Activation of the AhR was also recently shown to induce expression of $P27^{Kip27}$, a cyclin/cyclin-dependent kinase inhibitor, which was also associated with a delay in cell cycle progression through G_1 (*293*). These AhR-mediated effects on the cell cycle would appear to be entirely independent of the Ah battery of xenobiotic-metabolizing enzymes. It is hypothesized that a delay in cell-cycle progression would permit the cell time to repair oxidative or adductive damage that may have occurred as a result of elevated rates of P450-catalyzed metabolism. Recent studies have also indicated a role of the AhR in regulating apoptosis of human oocytes. The Bax gene, which

controls an apoptotic pathway, was shown to be Ah responsive, and it induced PAH but not by TCDD in oocytes (*294*). This AhR ligand specificity raises interesting questions as to the mechanism involved. It is possible that the effect requires metabolism of the ligand, possibly to a reactive intermediate. If this is the case, the effect would likely not be seen with some mixtures of AhR agonists.

Heavy Metal–Organic Toxicant Interactions

Heavy metals are often co-contaminants with organic toxicants in the environment. Thus, a number of studies have been initiated to investigate their toxic interactions at the molecular level (*295*). A significant component of the toxicity of a number of heavy metals, including cadmium, chromium, and arsenic, results from the fact that they are pro-oxidant; that is, they lead to generation of ROS and oxidative stress (*296*). The combination of metal-induced oxidative stress with other mechanisms of toxicity induced by organic toxicants is of significant concern. In addition, a number of organic toxicants including TCDD, PCBs, and PAHs may also induce oxidative stress (*167,297–299*). The effects of co-exposure to metals and organic toxicants may therefore be compounding. However, there appear to be some physiologic mechanisms by which the effects of pro-oxidant metals and organic toxicants are mutually abrogated at the molecular level. Our understanding of the metal–organic toxicant interactions has advanced significantly because of the recent elucidation of several intracellular stress–response signaling pathways and the points of cross-talk among them.

Pro-oxidant metals, and indeed, other toxicants that elicit oxidative stress, induce expression of a battery of genes whose functions appear to be to limit oxidation, protect cells from free-radical damage, and prevent neoplasia. These include, among others, heme oxygenase 1 (HO-1), the ferritin L-gene, expression of NQO1, γ-glutamyltransferases, and γ-glutamylsynthetase. Regulation of these genes occurs through antioxidant response elements (also referred to as electrophilic response elements) in the promoter regions of the genes (*300*); these are sites of interaction of the nuclear factor (Nrf) group of transcription factors (*301–303*). The HO-1 gene is well characterized with respect to induction by stresses and metals, including arsenic III, zinc, and cadmium (*304*). The role of HO-1 induction as a defense against oxidative stress is not hard to envision because heme is pro-oxidant, whereas bilirubin, the ultimate product of heme degradation, is an antioxidant. P450-catalyzed metabolism is recognized as a source of free radicals, notably when futile catalytic cycles occur with poorly metabolized substrates such as PCBs and TCDD (*305,306*). Coincident with the induction of HO-1 by arsenite is the reduction of the expression of P450s of several gene families (*307,308*). Although heme availability for the prosthetic group of P450 is undoubtedly at least partially responsible for the effect, there appear to be several pathways in which oxidant signals result in an effect on P450 expression.

We have considerable evidence of cross-talk involving stress signals and AhR-mediated gene transcription that may determine physiologic responses to environmental mixtures. We have known for some time that oxidative stress causes a down-regulation of the AhR-regulated P450s CYP1A1 and CYP1A2 (*309,310*). Recently, pro-oxidant metals cadmium, chromium, and arsenic disrupted AhR-mediated induction of the CYP1A1 and NQO1 mRNAs in Hepa-1 cells (*295*). A potential mechanism of the effect involves the stress–response transcription factor nuclear factor-κB (NF-κB), which is activated by a number of stimuli, including oxidants (*311,312*). Low levels of arsenite activate NF-κB (*313,314*); however, higher levels inactivate NF-κB by binding to a critical cysteine (*315*). NF-κB activation leads to induction of a number of genes mediating inflammatory responses in immune cells, but NF-κB appears to have functions in other cell types and may control whether a cell undergoes, or is rescued from, apoptosis. A direct binding interaction of the AhR and the NF-κB subunit, RelA, has been demonstrated, and the interaction was shown to suppress AhR-mediated gene transcription (*258*). This role of NF-κB may explain the negative effect of oxidative stress on CYP1A1 inducibility, although we also have evidence for a role of NF-1 in this effect (*310*). The AhR–RelA interaction may also explain the effect of certain cytokines in reducing CYP1A inducibility (*316,317*). The AhR–RelA interaction may represent a mechanism by which oxidative stress resulting from P450 catalytic activity is minimized, similar to the induction of HO-1.

Summary

Toxicant Interactions

The discussion above obviously addresses only a small subset of the possible interactions of toxicants at the molecular level. Figure 2 depicts some of the signaling pathways that may be affected by chemical mixtures, and the ways in which they may interact, based on the current literature. Even when we consider a limited number of signaling pathways, the potential not only for additivity and synergism but also for antagonism of toxicity is clear (*318*). It is apparent that stress–response pathways interact to enhance cellular defense mechanisms and limit metabolism that potentially leads to cellular damage.

Individual Susceptibility to Toxicant Mixtures

Susceptibility of an individual to the toxic and carcinogenic effects of a chemical mixture is believed to be affected to a significant

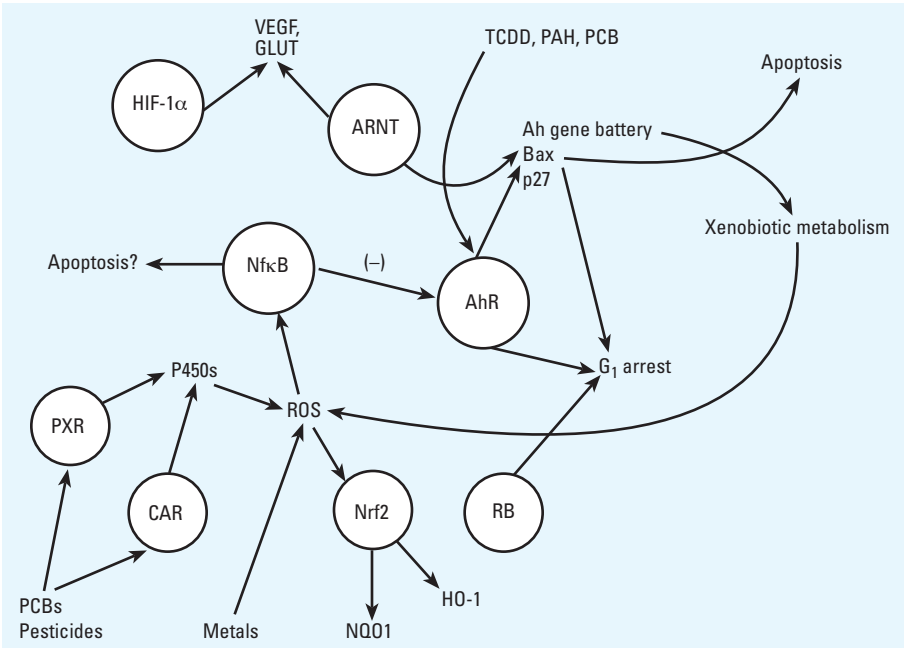

Figure 2. Signaling pathways and toxicant interactions. Glut-1, glucose transporter 1; Nrf2, nuclear factor-drythrod 2-related factor 2; RB, retinoblastoma protein; VEGF, vascular endothelial growth factor.

degree by genetics. Identification of alleles of specific genes associated with increased risk of diseases such as cancer is an ongoing process (*266,319,320*). However, susceptibility to an environmentally induced disease may involve not a single gene but rather a series of genes, some having a major effect on susceptibility, others having a modifying effect (*321*). For example, cancer susceptibility associated with a gene affecting the rate of metabolic activation of a procarcinogen could be highly influenced by the nature of genes encoding phase II detoxifying enzymes for reactive metabolites and DNA repair enzymes responsible for removing adducts (*9,322*). The advent of new gene array technology will not only allow the analysis of global patterns of gene expression in response to xenobiotic/mixture exposure (*323–325*) but also could eventually allow the genomewide identification of polymorphisms and mutations that may be used to predict an individual's response to drugs (*326*) or other xenobiotics. These new approaches of pharmacogenomics and toxicogenomics will undoubtedly be of paramount importance in unraveling the complex interactions among environmental toxicants and in determining their effects on human health.

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