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CanTox Inc., Consultants in Toxicology, Health, and Environmental Sciences, 308-2233 Argentia Road, Mississauga, Ontario, Canada L5N 2X7

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PREFACE

This report describes a number of important scientific principles that govern the evaluation of the potential for chlorinated organic chemicals to cause adverse effects on the environment and human health. The report is not intended to be a comprehensive evaluation of available data pertaining to chlorinated organic chemicals. Rather, certain of the published data reporting adverse effects are examined in light of the scientific principles elaborated to underline the point that, as in any branch of science, rigorous criteria must be applied in assessing the significance of the scientific data on chlorinated organic chemicals.

The report was reviewed by a panel of scientists chosen for their expertise in human health and environmental toxicology. John Giesy, Ph.D., Michigan State University, and Martin van den Berg, Ph.D., University of Utrecht, provided critical review in the area of environmental toxicology. Dietrich Henschler, M.D., Institut für Pharmakologie und Toxikologie der Universität Würzbürg, and Renate D. Kimbrough, M.D., Institute for Evaluating Health Risks, provided critical review of human health aspects of the report.

ABSTRACT

The term chlorinated chemicals is used to describe diverse groups of chemicals of varying chemical structure, including those used in water disinfection, as well as numerous aliphatic, aromatic, and polycyclic chlorinated substances. This report elaborates a number of scientific principles that govern the evaluation of the potential for chlorinated organic chemicals to cause adverse effects on the environment and to human health. The purpose of the report is to demonstrate the importance of applying these scientific principles in the evaluation of potential adverse effects of chlorinated

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organic chemicals. The four major principles upon which such a scientific analysis must be based are:

(1) the fate and biological activity of a compound are determined by the chemical properties of the compound;
(2) compounds do not show adverse effects below certain threshold concentrations, and the magnitude of response is related to dose;
(3) inherent metabolic processes allow organisms to accommodate low doses of chlorinated organic chemicals;
(4) observations associated with the presence of a certain compound must be biologically plausible effects, based on the specificity of the compound's activity in experimental systems.

With respect to the first of these principles, there is abundant scientific evidence that the physical and chemical properties of chlorinated organic chemicals govern their bioaccumulative potential, toxicological properties, and thus their potential behavior and effects in the environment. Chemicals that have low solubility in water are highly lipophilic and have low vapor pressure, tend to accumulate in biological systems, and degrade slowly in the environment. Chlorinated organic chemicals that possess these characteristics include those having a carbon ring structure and multiple chlorine substitution. Other chlorinated organic chemicals with lesser degrees of chlorine substitution, such as 2,4-dichlorophenoxyacetic acid (2,4-D), trichlorophenol, chloroform, and dichloroethane, do not share the physical and chemical properties of the high molecular weight, cyclic, polychlorinated compounds and, as such, do not have the same potential to bioaccumulate. These differences among chlorinated organic chemicals with respect to their physical and chemical properties and behavior in the environment preclude the generalization that all organic chemicals containing chlorine behave similarly in the environment and act as persistent, bioaccumulative chemicals.

The reactivity of chlorinated organic chemicals and hence their potential to produce biological effects depends on their specific molecular features. The substitution of chlorine into an organic molecule may increase or may reduce its biological activity. One determinant of biological activity is whether or not chlorinated and nonchlorinated organic chemicals can form electrophilic moieties which can react with critical targets in biological systems. Other chlorinated and nonchlorinated organic chemicals may interact with cellular receptor systems, resulting in changes in homeostatic functions through a variety of secondary effects. These are not properties shared by all chlorinated organic substances because not all are capable of being metabolically activated to reactive substances or of interacting with cellular receptor systems critical to the maintenance of normal functions.

A second important principle to be considered in assessing the significance of chlorinated organic chemicals in the environment is that of the dose–response relationship. This relationship dictates that the magnitude of the response of biological systems to chemicals depends on their concentrations in organisms, which in turn depend on the magnitude and duration of exposure. Adverse effects are observed when the rate of damage is greater than the rate of repair of cellular components. The magnitude of exposure is proportional to concentrations in the environment. The chlorinated compounds with greatest potential for bioaccumulation, such as some polychlorinated biphenyls (PCBs), some chlorinated dioxins and furans, 1,1,1-trichloro-2-2-bis(parachlorophenyl)ethane (DDT), dieldrin, and other structurally similar com-

pounds, act through threshold-type mechanisms. This means they would not be expected to elicit a toxic response at environmental concentrations which result in exposures below a threshold. National regulatory authorities have acknowledged the existence of environmental concentrations below which there would be no cause for concern, and this concept provides the basis for existing regulatory standards. The existence of thresholds for effects has been validated for the chlorinated organic chemicals, and adverse effects would occur only when these threshold exposures are exceeded. For example, in the Great Lakes, where bioaccumulative chlorinated organic chemicals as a group have been associated with reproductive effects and population declines of certain species of piscivorous birds, decreasing concentrations of representatives of these groups of chemicals have been associated with recovery in most species, particularly herring gulls and double-crested cormorants. Currently, reported adverse effects on piscivorous bird populations in the Great Lakes Basin are limited to localized areas of greatest environmental concentrations, such as Green Bay in Lake Michigan and Saginaw Bay in Lake Huron.

Further evidence of a threshold concentration for chlorinated organic chemicals below which adverse effects on the environment do not occur comes from the fact that to date approximately 1500 of these substances are known to be produced by natural processes. Chlorinated organics are released into the environment by a number of organisms, including bacteria, fungi, algae, ferns, and higher plants, by decomposition of seaweed, and by combustion sources such as forest and grass fires and volcanic activity. Naturally produced chlorinated organic chemicals include chlorinated alkanes, alkenes, phenols, polychlorinated biphenyls, and chlorinated dioxins and furans, encompassing a broad range of substances representing the same classes of chemicals as produced by human activities. Natural processes are known to result in significant background concentrations of some of these chemicals; however, the available information is inadequate to estimate total environmental releases for most chlorinated organic chemicals. Many of the chlorinated and other organic chemicals are metabolized through the action of the mixed function oxygenase (MFO) system, a nonspecific enzyme system present in diverse species from different phylogenetic classes. The ubiquity of chlorinated organic chemicals in the environment and the presence in diverse groups of mammalian and nonmammalian species of a common enzyme system capable of detoxifying these compounds provide evidence that organisms have evolved in the presence of such chemicals. The existence of the naturally occurring chlorinated organics supports the conclusion that exposures to sufficiently low concentrations of similar anthropogenic chemicals would not result in the occurrence of adverse effects on ecosystems.

The final principle which governs the assessment of the potential adverse effects of chlorinated chemicals in the environment is that of the specificity and biological plausibility of associating reported adverse effects with possible causal agents. Since chlorinated organic chemicals have diverse chemical structures, and therefore vastly differing bioaccumulative and toxic potentials, it is critical to use sound scientific judgment to establish associations between adverse environmental effects and causative agent(s). The establishment of cause–effect relationships is essential to invoke the development of effective mitigation procedures for improvement and protection of the environment.

The application of the principle of specificity of association is demonstrated in the following three examples. Mass mortalities of marine mammals have been alleged to be associated with exposures to chlorinated organic chemicals; however, the application

of criteria for the establishment of causal associations leads to the conclusion that natural disease processes are often to blame. The weight of available evidence appears to support an association between adverse effects on reproduction of fish-eating birds in the Great Lakes region and historical exposures to PCBs and DDE (the principal metabolite of DDT), although other less studied factors including mercury and other chemicals, habitat destruction, and natural diseases also are involved. In the case of pulp and paper effluents, complex mixtures of chlorinated and nonchlorinated chemicals have been implicated in cause–effect relationships between rates of chemical release and adverse effects on the aquatic environment. The results of more rigorous studies in this area indicate that the effects of pulp mill effluents cannot be attributed solely to the release of process-related chlorinated chemicals into the environment. Similar effects on fish are observed near mills that use or do not use chlorine, and significant reductions in the use of chlorine at other mills have not resulted in corresponding reductions of environmental effects. Similarly, the application of criteria for judging causal associations of human health effects leads to the conclusion that, except for well-documented instances of toxicity related to specific incidences of accidental releases and certain defined occupational exposures, there have been no definitive cause–effect relationships established between exposure to environmental concentrations of chlorinated organic chemicals and adverse health consequences in the human population.

It is clear, from the available scientific information, that chlorinated organic chemicals possessing the physical and chemical properties that lead to bioaccumulation have the greatest potential to produce adverse effects on the environment. It is also clear, because environmental concentrations of these compounds have decreased during the past 20 years, that animal populations previously thought to be affected by such chemicals are recovering, consistent with the principle that sufficiently small environmental concentrations of these compounds will not adversely affect the environment. This principle also is supported by the fact that naturally occurring sources are responsible for significant releases of chlorinated organic chemicals, including some having a high degree of chlorine substitution. While the significance of natural sources of chlorinated organic chemicals to environmental health is not well understood, the available evidence indicates that the earth's species have evolved with metabolic capability to accommodate small concentrations of these substances.

INTRODUCTION

The purpose of this report is to provide a discussion of scientific principles governing the evaluation of the potential for chlorinated organic chemicals to produce adverse effects on the environment and human health. Their potential to produce adverse effects has been the focus of a continuing debate among environmental groups, governments, and industry. The term "chlorinated organic chemical" describes a broad range of chemicals of diverse chemical structure, ranging from simple aliphatic substances to complex chlorinated aromatic and polycyclic substances. Also included are many substances of medicinal and health importance such as pharmaceuticals, pesticides, and disinfectants.

Available information demonstrates that physical, chemical, and biological properties of chlorinated organic chemicals determine their behavior in the environment

and their potential effects on the ecosystem. Information on specific physical/chemical properties of chemicals has proven reliable in predicting their behavior or fate in the environment (Mackay *et al.*, 1992; Klopman *et al.*, 1993). In addition, these properties of chemicals influence the metabolism of chemicals by organisms (including humans) and this, along with the level of exposure (dose), determine their potential to produce toxicity. However, physical/chemical properties have proven to be less reliable predictors of adverse effects than of environmental fate (Henschler, 1990).

The association of bioaccumulation with specific types of chemical structures is well documented. For example, it has been established that increased concentrations in tissues of certain polychlorinated biphenyls (PCBs) or 1,1,1-trichloro-2,2-bis(parachlorophenyl)ethane (DDT) have been linked to adverse responses (Tillitt *et al.*, 1992). The occurrence of elevated concentrations of such chemicals in tissues is principally due to their potential to biomagnify in aquatic food chains (Environment Canada, 1991a). The bioaccumulation of these substances results from their molecular stability coupled with their greater solubility in fat compared to water (McEwen and Stephenson, 1979). These properties of stability and lipid solubility are not general properties of all chlorinated organic substances. Most chlorinated chemicals are degraded rapidly by inherent processes common to numerous organisms in the environment. Furthermore, the view that chlorinated organic chemicals are singularly a by-product of human activities is not borne out by an examination of the scientific literature which demonstrates conclusively that a broad array of chlorinated chemicals is produced naturally in the environment (Gribble, 1992). Importantly, enzymatic processes capable of metabolizing these substances are present in organisms in the environment (Environment Canada, 1991c).

An additional factor that warrants consideration in any examination of the potential adverse effects of chlorinated substances concerns their concentrations in various environmental media. Adverse biological effects are dose-related, and the contention that the presence of a chlorinated chemical in the environment is an adequate basis to conclude that adverse consequences will occur is not supported by the application of the scientific principles of toxicology. In this report, available data concerning reported adverse effects of chlorinated organic chemicals are examined in light of the concept of dose–response principles. The report also makes note of the fact that, except for some continuing "hot spots," the concentrations of chlorinated organic chemicals in environmental media and organisms have declined considerably since the 1970s, thus reducing the potential for future risk.

An additional important consideration in the evaluation of the potential for chlorinated organic chemicals to cause adverse effects is the adequacy of data supporting the relationships between the presence of these substances in the environment and reported adverse consequences. As with any chemical, the toxic potential of chlorinated organic chemicals to organisms depends on their chemical structure, pharmacokinetics and metabolism, and interactions with cellular systems. Thus, the potential for adverse effects of substances within the varied classes of chlorinated chemicals must be evaluated taking these factors into consideration. The strength of associations between the concentrations of chlorinated organic chemicals and reported adverse effects warrants careful examination in light of the quality and quantity of available data. Evidence for adverse effects is strengthened by the availability of laboratory data demonstrating dose–response relationships for individual chlorinated substances, while field data

demonstrating only statistical associations must be critically examined before scientific judgments of causal associations are reached.

Keeping in mind these important concepts of physical, chemical and biological properties, dose–response relationships, natural occurrence and applicable metabolic processes, and specificity of association of reported adverse effects, what follows is a critical examination of how these principles relate to the evaluation of the potential for chlorinated chemicals to cause adverse effects in the environment, including human health.

PHYSICAL AND CHEMICAL PROPERTIES OF CHLORINATED ORGANIC CHEMICALS

Chlorinated chemicals comprise structurally diverse groups of chemicals with a broad spectrum of physical and chemical properties. Chlorinated compounds are represented in all the major chemical classifications including inorganic salts and acids, monocyclic, polycyclic, heterocyclic, simple and branched chain aromatics, and aliphatics. The physical and chemical properties of chemicals, including chlorinated organic chemicals, govern their behavior in the environment and profoundly affect their activity on biological systems.

The reactivity of organic molecules depends on the structural features of their carbon backbone (e.g., straight chains, cyclic, polycyclic) and the nature, number, and position of functional groups attached to this backbone. In the basic or parent unsubstituted organic chemical, hydrogen atoms are attached to the carbon atoms. The hydrogen atoms can be replaced by various functional groups (e.g., oxy-, hydroxyl-, carboxy-, alkyl-, allyl-, alkoxy, nitro-, amino-, sulfoxy-, sulfhydryl-, chloro-, bromo-, iodo-, fluoro-groups plus many others). The introduction of various functional groups changes the physical and chemical characteristics of the parent organic molecule by changing its molecular weight, size, shape, and electrical and reactive properties (Politzer and Laurence, 1984). These changes in molecular properties by the addition of various functional groups to organic chemicals are determinants of the interactions of these chemicals with biological systems.

Overall, the properties conferred by the addition of different functional groups to organic molecules are part of a continuum, with the intensity of change in properties varying with type, number, and positioning of the functional group(s) added to the molecule. Hence, significant changes may be observed in the physical/chemical parameters between substituted and nonsubstituted parent molecules (Verschueren, 1983). Molecular volume is a major factor affecting the physical/chemical parameters of organic molecules. For example, the effects on molecular volume of the addition of one atom of chlorine are approximately the same as the addition of one methyl (CH_3) group (Mackay *et al.*, 1992). Therefore, the substitution of a hydrogen atom by one atom of chlorine or by one methyl group would result in approximately the same change in vapor pressure, Henry's law constant, water solubility, and octanol/water partition coefficient of an organic molecule although different substituents would impart different degrees of reactivity (Mackay *et al.*, 1992). The scientific understanding of how the addition of various functional groups alters the physical/chemical properties of chemicals is now considered to be sufficient to allow judgments to be made regarding likely behavior in the environment of specifically designed molecules, thus providing

a process for identifying and then avoiding releases of those with undesirable characteristics that would result in unwanted environmental distribution and deposition, with possible adverse effects (Mackay *et al.*, 1992).

Predictions of Behavior and Fate of Chlorinated Organic Chemicals in the Environment

Predictions of fate and bioaccumulation of chemicals in the environment based on their physical/chemical properties have been quite successful. Water solubility and octanol–water partition coefficient (log K_{ow}) are measures of chemical solubility in water and fat, respectively. Vapor pressure is a measure of the extent to which a chemical evaporates from soil or water into the air. For example, the physical and chemical properties of PCBs indicate that these compounds are not very soluble in water (Mackay *et al.*, 1992). Therefore, in an aquatic environment, PCBs partition to the organic phase (e.g., sediments, biota) to a much greater extent than to the aqueous or water phase. The greater their degree of chlorination, the greater the tendency to accumulate and persist in soils and sediment that have greater organic carbon concentrations, and the greater their capacity to bioaccumulate in aquatic and terrestrial wildlife and demonstrate substantial food chain biomagnification (Environment Canada, 1991a).

The physical/chemical properties of the majority of the chlorinated organics in use by society today are substantially different from those of the higher chlorinated PCBs. For example, the water solubility of chlorinated alkanes and alkenes range from 1100 to 9300 mg/liter (Verschueren, 1983). Correspondingly, log K_{ow} values for these chemicals are low and range from 1.45 to 2.73 (Hansch and Leo, 1979; Banerjee *et al.*, 1980; Valvani *et al.*, 1981). Because of their high water solubility and low solubility in lipid, there is negligible bioaccumulation of these chemicals in the environment.

Furthermore, chlorinated organic chemicals possess a range of vapor pressures. For example, 1,2-dichloroethane has a much greater vapor pressure compared to the vapor pressures of chlorinated dioxins (e.g., 8132.7 Pa at 25°C compared to 0.073 to 1.10 $\times 10^{-10}$ Pa) (Moore *et al.*, 1991). Thus, most of the 1,2-dichloroethane released into the environment volatilizes to the atmosphere, where it is degraded by interactions with hydroxyl radicals produced by natural sunlight (Clement Associates, 1989). As a result, this chemical would be expected to volatilize rapidly from surface waters (Clement Associates, 1989; Moore *et al.*, 1991). This behavior is predictable based on environmental fate modeling derived from physical/chemical properties, which indicate that the majority (about 97%) of 1,2-dichloroethane would partition to air, with only very small proportions being distributed in soil, sediments, and fish (Mackay *et al.*, 1992).

Differences in physical/chemical characteristics, which are the result of the degree and pattern of chlorination, among other parameters, influence the degradation of chlorinated organic chemicals in the environment. For example, PCBs without substitution in the *ortho* positions of the carbon rings, and with chlorine atoms in both *para* positions and at least one *meta* position, form "coplanar" structures where the carbon rings lie molecularly in flat planes. Some coplanar PCBs tend to bioaccumulate to a greater extent than noncoplanar PCBs in organisms higher in the phylogenetic

tree (Tillitt *et al.*, 1992). The presence of a significant degree of chlorination is not, of itself, sufficient to confer bioaccumulative potential on a compound. This is supported by the observation that moderately chlorinated aromatics, such as 2,4,5-trichlorophenol, with a log K_{ow} of 4.19 (Schellenberg *et al.*, 1984), are not particularly bioaccumulative. Chlorophenols are rapidly absorbed by aquatic invertebrates and fish; however, they are also rapidly metabolized and excreted and therefore generally do not bioconcentrate to the extent expected based on the log K_{ow}. Given the difference in physical/chemical characteristics, the behavior and fate characteristics associated with bioaccumulative compounds are not applicable to other chlorinated organic compounds with substantially different physical/chemical characteristics.

In applying the scientific principles that dictate the relationship between physical/chemical properties and biological effects and environmental fate characteristics, it is possible to make judgments as to the likely behavior and potential for adverse effects of various groups of chlorinated chemicals. For example, compounds having low water solubility, low vapor pressure, and high lipid solubility are generally very stable compounds under most environmental conditions. Upon introduction to the aquatic environment, PCBs and chlorinated dioxins readily partition into the sediment (Pavlou and Dexter, 1979; Phillips, 1986). Due to their hydrophobicity (low water solubility), these chemicals adsorb to dissolved organic material (DOC) and organic carbon of suspended particulates in the water column, dissolved organic material in the pore water, organic carbon of the bed sediments, and fatty tissues of aquatic biota. Lesser chlorinated PCBs and chlorinated dioxins/furans are more soluble in water (Haque *et al.*, 1974; Haque and Schmedding, 1976) and are expected be transported further than those with greater chlorine substitution (Phillips, 1986). The greater vapor pressure and lesser binding coefficient to particulate materials make the lesser chlorinated dioxins and furans more susceptible to degradation in the atmosphere by ultraviolet light (Eitzer and Hites, 1989; Hites, 1990). Since some PCBs, chlorinated dioxins and furans, and other highly chlorinated aromatics are relatively stable both thermally and chemically, environmental concentrations would be expected to change slowly with time (Chou and Griffin, 1986). This is further exacerbated by the continued release of PCBs from municipal landfills and various waste storage sites. In addition, there are releases of chlorinated dioxins and furans from numerous uncontrolled human activities (e.g., emissions from internal combustion engines used in automobiles and other applications, combustion of organic fuels for heating and other purposes) and an undetermined extent of release from naturally occurring sources (Bumb *et al.*, 1980; Nestrick and Lamparski, 1982; Clement *et al.*, 1985; Travis and Hattemer-Frey, 1989; Tashiro *et al.*, 1990). Thus, these chemicals have the potential to be an environmental problem in the future, despite their restricted or banned use in certain specific instances.

Many chlorinated organic chemicals can be degraded by various chemical reactions, including hydrolysis, photochemical oxidation, as well as biomediated oxidative and reductive reactions such as degradation by bacteria and fungi. A chemical may be resistant to one or more forms of degradation, but susceptible to other forms. For example, hydrolysis of chloroform has been reported to be a very slow process (Mabey and Mill, 1978; Jeffers *et al.*, 1989), but volatilization of chloroform from water occurs rapidly with a half-life of 1 to 31 days (Zoeteman *et al.*, 1980; SRC, 1989) and it is degraded in the environment by microorganisms (Tabak *et al.*, 1981; SRC, 1989). Conversely, chlorinated phenols have relatively low vapor pressures at environmental temperatures in the aquatic environment resulting in minimal volatilization (Pignatello

et al., 1983; Sugiura *et al.*, 1984; HWC, 1988). These chemicals are removed from the aquatic environment primarily through photolysis and through microbial transformation (Agriculture Canada, 1987). These examples illustrate the importance of degradation pathways in the natural environment.

Even among chemicals with aromatic structures, the presence of one or more chlorine atoms confers neither persistence in the environment nor a significant potential for bioaccumulation. For example, 2,4-dichlorophenoxyacetic acid, or 2,4-D, a chlorophenoxy herbicide, has an aromatic structure substituted with two chlorine atoms and does not persist under most soil conditions because it is readily metabolized in plants, soil, and in the aquatic environment by bacteria and fungi that adapt to 2,4-D, using it as a food source (McEwen and Stephenson, 1979; Stevens and Sumner, 1991). Consequently 2,4-D is rapidly degraded in the environment, with a half-life measured in days to weeks (McEwen and Stephenson, 1979). Although this chemical is aromatic and substituted with chlorine atoms, it is not a potent mammalian or avian toxicant (Munro *et al.*, 1992), and it has a very low potential for bioaccumulation. Thus, although PCBs, chlorinated dioxins and furans, 1,2-dichloroethane, trichlorophenol, chloroform, and 2,4-D are all chlorinated organic chemicals, differences in their physical/chemical properties cause them to behave differently in the environment. Clearly, the behavior and fate in the environment of one type of chlorinated organic chemical, such as highly chlorinated bioaccumulative compounds, cannot be used to predict the behavior and fate of all chlorinated organic chemicals. The striking differences in behavior and fate in the environment among individual chlorinated organic chemicals indicate that scientifically valid evaluations of the potential adverse effects from environmental concentrations of these chemicals must be based on specific data related to chemical and physical properties of individual compounds.

Predictions of Adverse Effects of Chlorinated Organic Chemicals

The predictions of the effects of adding functional groups on the biological activity of molecules have proven more difficult than predicting their influence on environmental behavior. The addition of a specific functional group with electronegativity and electron-withdrawing characteristics (e.g., chlorine and other halogens, NO_2, SO_3) to an organic molecule may increase or reduce the chemical reactivity of the molecule, depending on the position and number of substitutions, and the atoms replaced by the functional groups. This means that not all chlorinated chemicals are more chemically or biologically reactive than their nonchlorinated parent chemicals, and that the addition to the molecule of other atoms or moieties (e.g., NO_2, SO_3, NH_2, etc.) can result in the production of chemicals with greater reactivity than those containing chlorine. Other relevant factors are the presence of additional electronically active functional groups on the molecule and the chemical structure of the molecule itself (e.g., steric factors).

The electron-withdrawing properties of chlorine and other halogens are considered to be the main factors governing the reactivity of organohalogens (Huheey, 1965; Ternay, 1979) and in this regard, there are major differences among chlorinated organic chemicals. For example, the chemical reactivity of chloro- and haloalkenes is dependent on the nature, the number, and the position of halogen substituents, as well as the number and the position of the double bonds (Williamson and Cvetanovic, 1968;

Ternay, 1979; Woo *et al.*, 1985). In the ethylene series, introduction of the electronegative halogen atoms decreases the electron density in the double bond and exerts a stabilizing effect. Indeed, the reactivity of ethylene decreases dramatically with the increase in the degree of chlorination (Williamson and Cvetanovic, 1968). In the propene series, allyl halides (3-halopropenes) are expected to be much more susceptible to nucleophilic substitution of the halogen atom than the corresponding haloalkanes (Woo *et al.*, 1985). Vinyl halides are poor alkylators, and enzymatic activation is required, with vinyl bromide being slightly more potent than vinyl chloride (Barbin *et al.*, 1975; Eder *et al.*, 1980).

In contrast, allyl halides are good alkylators even without metabolic activation, their activity depending on the halogen substitute. Iodine-substituted allyl halides are strong alkylators, with bromine and chlorine being weaker (Eder *et al.*, 1980). The various factors that may affect this behavior have been summarized by Woo *et al.* (1985). The addition of greater quantities of chlorine to organic molecules tends to decrease their reactivity and increase their stability and bioaccumulative potential in the environment. Larger, more complex molecules with larger quantities of chlorine (e.g., PCBs, chlorinated dioxins and furans) are less likely to have reactive metabolites or if they do, the quantities formed are smaller than formed from lesser chlorinated chemicals.

Enzyme systems involved in the initial stages of metabolism of chemicals (i.e. Phase I metabolism) generally catalyze the formation of reactive nucleophilic and electrophilic metabolites of chlorinated organic chemicals that can interact with cellular macromolecules (e.g., DNA). For example, reactive metabolites of chlorinated alkyl benzenes, chlorinated nitrobenzenes, chloroform, and minor amounts of metabolites of PCBs and other chlorinated polycyclic compounds form through reactions with the cytochrome P450-containing monooxygenase enzyme system in the liver. These reactive metabolites can covalently bind to various cellular macromolecules, but they also react during Phase II metabolism with reduced glutathione (GSH) through the catalytic actions of the glutathione *S*-transferase enzyme system. The covalent binding of the reactive metabolites to GSH prevents their reaction with vital cellular macromolecules, thereby protecting cells from the toxic effects associated with these reactions (Sipes and Gandolfi, 1991).

The balance between the formation of reactive metabolites from chlorinated organic chemicals that undergo such reactions and their subsequent inactivation by GSH is critical to the potential expression of adverse effects from such chemicals. Imbalance in the glutathione *S*-transferase–GSH conjugation system can substantially affect the toxic potential of chemicals that produce toxicity via reactive metabolites. In addition, large quantities of reactive metabolites (e.g., as may be observed with extreme exposures used in laboratory tests) can deplete the available concentrations of GSH in cells. Since GSH is a cofactor for various normal cellular reactions, an array of secondary toxic effects can result from its depletion (e.g., GSH is a cofactor for glutathione peroxidase, and depletion of GSH can result in increased lipid peroxidation leading to a variety of toxic effects) (Sipes and Gandolfi, 1991). These considerations shed doubt on the validity of extrapolating information from extreme exposures (e.g., those typical of most laboratory tests, or associated with large quantity accidental releases of chemicals into the environment) to lesser exposures more typical of those related to usual environmental pathways where GSH depletion would not occur.

Chemical structure also has an important bearing on the nature of toxicity. Except for narcosis, which is a nonspecific, high-dose effect of most chlorinated and nonchlorinated organic substances (Franks and Lieb, 1982; 1984), molecular configuration greatly affects the specific form of toxicity. An example of this phenomenon can be drawn from knowledge of chemical carcinogenesis. Evidence to date indicates that carcinogenic chemicals can be divided into two major classes, genotoxic carcinogens and epigenetic or nongenotoxic carcinogens (Williams and Weisburger, 1991).

(i) Genotoxic carcinogens chemically interact with DNA, and adduct formation is a definitive way to identify genotoxic carcinogens. Genotoxic carcinogens are subdivided into those where the parent chemical is active and those that require bioactivation where a metabolite is the active form.

(ii) Epigenetic or nongenotoxic carcinogens do not interact with genetic material, but act via an indirect biological mechanism as the basis for their carcinogenicity. Epigenetic carcinogens function as promoters, modifiers of hormonal activity, cytotoxic agents, immunosuppressors, peroxisome proliferators, etc. Many of the carcinogens in this class increase DNA synthesis, mitosis, and cell replication rates following extreme exposures. Some produce secondary genotoxic effects through modifications in other cellular and metabolic systems.

These properties are important in assessing the carcinogenicity of chlorinated organic chemicals (Henschler, 1985), and the distinction of the two classes is critical to risk assessment. Genotoxic carcinogens represent a threat to human health because of the interactions with genetic material and because of the possibility that they can act after a single exposure or in a cumulative manner with multiple exposures. Thus, more stringent criteria are required to establish acceptable or allowable exposures to genotoxic carcinogens (OSTP, 1985; Williams and Weisburger, 1991). Epigenetic carcinogens, on the other hand, show carcinogenic effects only following extreme and sustained exposures that are necessary to maintain abnormal physiological functions, hormonal imbalance, tissue injury, cell proliferation, or other nongenetic mechanisms (Williams and Weisburger, 1991). Exposures that do not produce the required alterations in physiologic, homeostatic parameters that are affected by the epigenetic carcinogen would not result in an increased incidence of cancers. Therefore, for epigenetic carcinogens it is possible to establish exposure thresholds below which cancers would not develop (Williams and Weisburger, 1991).

While a great deal is known about the relationship of chemistry and mechanisms of carcinogenesis, it has proven difficult to predict the carcinogenicity of chemicals based on their specific molecular structures (Henschler, 1990). This difficulty is believed to be related to the complexity of carcinogenesis, which involves many biological processes, including uptake, metabolism, distribution, excretion, and specific interactions of chemicals with cellular macromolecules (e.g., DNA). For example, cleavage of GSH conjugates of chemicals by β-lyase in the kidney results in the formation of reactive metabolites that can lead to toxicity and cancer (Vamvakas *et al.*, 1993).

Detailed QSAR analyses of large carcinogenicity databases have shown that structural elements are not related consistently to carcinogenicity (Chou and Jurs, 1979; Jurs *et al.*, 1979; Craig and Enslein, 1981; Enslein and Craig, 1982; Rose and Jurs, 1982; Klopman, 1984; Klopman *et al.*, 1985; Frierson *et al.*, 1986; Ashby and Tennant, 1991). Among compounds containing "structural alerts," a large proportion were not carcinogenic, and 27 compounds not containing structural alerts did result in an in-

...ased tumor incidence in laboratory rodents (Ashby and Tennant, 1991). Despite the difficulties in making generalizations about specific molecular characteristics and the development of cancer, empirical observations demonstrate that the presence of chlorine in a molecular structure does not necessarily render the compound carcinogenic. In fact, the opposite is true in the case of benzene, a potent chemical carcinogen which has been associated with leukemia in humans at sufficiently great exposures but which has several chlorinated derivatives that are not carcinogenic (Ashby and Tennant, 1991). Some chlorinated compounds might be expected to show carcinogenic activity by virtue of their structures, while they are actually noncarcinogens. Examples are 2-chloroacetophenone, a compound containing a reactive chlorine, and chloroethane, a potent mutagen with a great degree of alkylating activity (Ashby and Tennant, 1991). Some chlorinated epoxides, compounds which might be expected to be carcinogenic based on their structure, are indeed not carcinogenic. For example, ethylene oxide is carcinogenic, while its di-, tri-, and tetrachlorinated derivatives are not (Politzer and Laurence, 1984). On the other hand, propylene oxide and its chlorinated derivatives, 1-chloropropylene oxide and 1,3-dichloropropylene oxide, all cause increased cancers in test animals (Politzer and Laurence, 1984). Among a large group of electrophilic compounds tested in the National Toxicology Program, 17/30 (56%) of the positive carcinogens contain a halogen (i.e., chlorine, bromine, iodine, or fluorine) substituent, while 4/11 (36%) of the chemicals that were not associated with increases in tumors contain a halogen. Among compounds containing a nonreactive halogen, 26 were positive and 16 were negative in cancer bioassays, and 8 are considered equivocal (Ashby and Tennant, 1991). These empirical data demonstrate that the presence of chlorine in a molecule does not necessarily impart carcinogenicity, and that specific testing is needed to assess the carcinogenic potential of chlorinated organic chemicals.

In summary, the information now available demonstrates that physical/chemical properties and chemical structures can be used to predict the environmental behavior of chemicals with reasonable confidence. On the other hand, less confidence can be placed in the predictions of specific toxicity, although various structural alerts and critical assessment procedures (e.g., adduct formation) can provide important clues about the potential toxicity of chemicals.

DOSE–RESPONSE RELATIONSHIPS

In evaluating the significance of environmental concentrations of chlorinated organics, it is of critical importance that principles governing dose–response relationships be considered (Klaassen and Eaton, 1991). The presence of chlorinated organic chemicals in the environment (regardless of their concentrations or specific chemical structures) does not in itself constitute a risk of adverse effects, in keeping with a fundamental premise of toxicology that effects are related to dose.

In addition, in the case of most if not all chemical compounds, there is a dose below which no adverse effects can be observed. Known as the threshold dose, its magnitude varies for different chemicals and varies for different organisms exposed to the same chemical. Factors that determine the threshold dose include the extent to which the chemical is absorbed by the organism, the rate and mechanism of clearance from the organism, the extent of metabolic production of toxic metabolites and/or detoxification products, and the degree to which the organism can repair any cellular damage by the

chemical. The ability of the organism to repair damage is a critical consideration when judging the applicability of the extrapolation information obtained from genetically inbred strains of animals to general heterogenetic populations. In establishing a safe level of exposure to chlorinated chemicals in the environment, empirical evidence of safety based on threshold doses observed in controlled studies must be considered, in addition to mechanistic evidence which helps to explain the basis for the existence of the threshold.

In this section, the application of the threshold concept in regulatory decision making is discussed, and several examples illustrating the operation of threshold and dose–response principles in the natural environment are presented. For acute or lethal toxic effects, the existence of an exposure threshold below which lethality would not occur (i.e., zero probability of a response) has long been recognized (Klaassen and Eaton, 1991). For most chronic adverse effects, there is generally a discernible threshold below which it is not possible to measure adverse effects in exposed populations; even though the absence of a threshold cannot be proven for certain endpoints.

The fundamental basis for the assessment of potential adverse effects on the environment and subsequent regulatory decision making is that the magnitude of a response of an organism to a chemical increases as the dose increases, and this dose–response relationship is based on three key observations (Klaassen and Eaton, 1991):

(i) A direct biological effect from a chemical can occur when the chemical causing the effect is present in the tissues of the organism where the toxic effect occurs. Secondary effects at other sites can occur that are triggered by the direct effect;

(ii) Once the concentration of the chemical in the tissues reaches a critical value that exceeds the ability of the tissue to counteract the effects of the chemical, the magnitude of the effects on the organism then increases in proportion to the concentration of the chemical in the tissues;

(iii) The amount of chemical at the site of toxic action in the tissues is proportional to the amount of chemical absorbed into the organism, and the amount of chemical absorbed depends, in part, on the concentration of the chemical in the environment to which the organism is exposed. The consideration of the bioavailability of the chemical is important in the evaluation of potential adverse effects, since many chlorinated organic chemicals in complex environmental media are absorbed much less efficiently than from ideal test vehicles used for administration in laboratory studies.

In keeping with the above observations, regulatory processes are designed to reduce risks to acceptable levels, recognizing that the theoretical ideal of "Zero Risk" is not practical nor is it attainable (Johannsen, 1990). The existence of threshold doses for numerous potentially toxic substances has been acknowledged by the American Conference of Governmental Industrial Hygienists (ACGIH) in its threshold limit values, by the FAO/WHO Joint Expert Committee on Food Additives, and the FAO/WHO Joint Meeting on Pesticides in their establishment of acceptable daily intakes (ADI) for hundreds of food additives and pesticide residues, and by regulatory agencies in various countries around the world, in the development of regulatory standards for numerous and diverse types of chemicals encountered in food, consumer products, and the environment.

Chlorinated organic chemicals are generally agreed to have exposure thresholds for adverse effects, for example, PCBs (EPA, 1988), chlorinated dioxins (Kociba *et al.*, 1978), DDT, and dieldrin (Newton *et al.*, 1989), all of which elicit a toxic response

at or above a threshold tissue concentration or dose. A threshold for adverse effects does not mean that reversible changes in various physiological and biochemical parameters, occurring without adverse consequences to the organism, would not be observed at small exposures (Klaassen and Eaton, 1991). For example, a proposed mechanism of liver tumor production by chlorinated dioxins is through a combination of their cytotoxic effects on liver cells and indirect effects which cause imbalances in the endocrine system due to the induction of enzyme systems involved in hormone metabolism. At doses below those resulting in liver cell toxicity and hormonal changes, no carcinogenic effect is expected. Similarly, thresholds would exist for adverse effects on reproduction, immune function, and other effects. Many of these adverse effects are secondary to changes in enzyme activities induced by chemicals (e.g., induction of aryl hydrocarbon hydroxylase (AHH) enzyme systems by chlorinated dioxins). These enzyme systems also change activities as part of normal physiological responses; therefore, merely inducing the enzyme does not necessarily mean an adverse effect will occur. In Canada, regulatory agencies have based their evaluation of the potential risk of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the fact that practical thresholds exist for tumorigenesis and reproductive effects, and have set an exposure limit of 10 pg/kg/day (CEPA, 1990) with the recognition that a safe exposure exists, below which there would be no expectation of adverse effects. Other countries, including Norway, Sweden, the United Kingdom, and Germany also regulate 2,3,7,8-T_4CDD as a chemical with a threshold-type mechanism of action; however, the safety factors used in establishing maximum allowable exposure limits vary from 100-fold, as used in Canada, to 1000-fold as used in Germany.

Mounting evidence supports the premise that there is a practical threshold for adverse effects. For example, as concentrations of groups of bioaccumulative chemicals in the environment have decreased, species of wildlife previously affected by historically greater concentrations are returning to normal health status, even in the presence of lesser concentrations of these groups of chlorinated organic chemicals. These findings support the regulatory premise that thresholds exist below which adverse effects would not be expected to occur, and that the presence of a chemical in the environment does not necessarily result in toxic effects. In the remainder of this section, examples of this dose–response phenomenon and evidence for the existence of a threshold in reported of dose responses in exposed wildlife will be presented.

The importance of considering dose–response relationships in the evaluation of chlorinated organic chemicals is demonstrated by the available literature relating dose to effect for specific chlorinated organic chemicals. In the past, exposures to environmental concentrations of certain bioaccumulative chlorinated organic chemicals have been sufficiently great to implicate these chemicals in the reproductive failure and birth deformities that have been observed in several species of colonial piscivorous birds inhabiting the Great Lakes Basin (Yamashita *et al.*, 1993; Hoffman *et al.*, 1987; Kubiak *et al.*, 1989; Fox *et al.*, 1991; Gilbertson *et al.*, 1991; Ankley *et al.*, 1992; Tillitt *et al.*, 1991, 1992). Decreased reproductive performance and/or population declines were reported through the 1960s and 1970s in at least nine top-predator bird species in the Great Lakes and other regions of North America. These declines have been associated with increased tissue concentrations of several bioaccumulative chlorinated organic chemicals (e.g., chlorinated dioxins/furans, PCBs, hexachlorobenzene, lindane, DDE, dieldrin), although the simultaneous destruction of necessary habitat and exposure to an array of other nonchlorinated chemicals and metals are also likely involved

While exposure of affected species to these bioaccumulative chemicals has decreased in recent years, and several species have experienced recovery, some species have not yet fully recovered. Developmental and reproductive effects still are observed in the areas of the Great Lakes Basin (Environment Canada, 1991a; Tillitt *et al.*, 1991, 1992) and Western Europe (van den Berg *et al.*, 1992; Bosveld *et al.*, 1992) where the greatest concentrations of these chemicals and loss of habitat due to various human activities occur. These observations suggest that localized exposures are above the thresholds for adverse effects. In other areas of the Great Lakes Basin, the environmental concentrations of these chemicals appear to have decreased such that exposures are below the threshold and no longer produce measurable adverse effects (Fox *et al.*, 1991).

Another manifestation of the dose–response relationship observed in populations of wild birds is the decline and recovery of the bald eagle associated with the rise and fall of environmental concentrations of DDT and its metabolites. As a tertiary predator, the bald eagle may be exposed to the greatest concentrations in its food and receive the greatest dose of such bioaccumulative chemicals of all wildlife. It is believed that exposures to DDT and its metabolites (and possibly other bioaccumulative chemicals) were associated with severe population declines of bald eagles on the shores of Lakes Ontario and Michigan (Gilbertson *et al.*, 1991), although loss of habitat was also a likely factor. The current reproductive status of Great Lakes bald eagle has greatly improved in several regions, with estimates of breeding pairs at 2400 in 1989 compared to a low of 400 breeding pairs in 1964. Although populations of bald eagles have not yet recovered on Lakes Huron and Ontario, the Lakes Erie and Superior populations now appear to be breeding normally. The lack of suitable habitat (e.g., loss of physical habitat, excessive human populations, large urban areas) may also have an important role in the poor reestablishment of bald eagles, particularly in some areas of the lower Great Lakes (Grier, 1982).

Similar situations have been observed in other piscivorous bird populations around the Great Lakes (Struger and Weseloh, 1985; Noble and Elliot, 1986; Gilbertson *et al.*, 1991). Increases in concentrations of certain chemicals in their tissues have been associated with population declines. Subsequent reductions in concentrations of chemicals have been associated with population recoveries. By the early 1960s, the double-crested cormorant had essentially ceased reproducing in Lakes Michigan and Ontario, and populations were also declining in the other Great Lakes (Environment Canada, 1991b). In 1972, 95% of the eggs laid in the Georgian Bay colony disappeared or were broken by the end of the incubation period (Environment Canada, 1991c). Cormorants are particularly sensitive to eggshell thinning, an effect associated with DDE (Tillitt *et al.*, 1992), since they incubate their eggs by standing on them (Environment Canada, 1991c). Between 1979 and 1987, the incidence of bill defects in cormorants in the Green Bay (52.1 per 10,000) and Beaver–Mackinac (12.3 per 10,000) areas of Lake Michigan were substantially greater than in Lake Superior, Huron, or Ontario, in Northern Ontario, or in the Canadian Prairies (Fox *et al.*, 1991). It has been postulated, based on a weight-of-evidence assessment, that the bill deformities observed in cormorants were associated with elevated tissue concentrations of certain bioaccumulative chlorinated chemicals such as the non-*ortho*-chlorine substituted dioxins, furans, and PCBs (Fox *et al.*, 1991; Environment Canada, 1991c). This postulate is based on observations that some of the non-*ortho*-chlorine-substituted isomers in the tissues of these birds are known to cause abnormalities in skeletal development

in experimental laboratory animals (Courtney *et al.*, 1976; Marks *et al.*, 1981; Wardell *et al.*, 1982; EPA, 1985; MOE, 1985; Weber *et al.*, 1985).

In keeping with the principle that response is a function of dose, decreased concentrations of persistent chlorinated organic chemicals in the Great Lakes were associated with a 56% annual increase in double-crested cormorant populations between 1974 and 1982 (Price and Weseloh, 1986). Current populations are greater than at any other time since cormorants entered the region in about 1928 (Environment Canada, 1991a). The adverse reproductive effects now observed are confined to a few greatly contaminated areas, such as Green Bay in Lake Michigan, where the occurrence of crossed bills in cormorants remain elevated (Tillitt *et al.*, 1991, 1992).

The above discussion indicates that developmental effects, impaired reproduction, or complete reproductive failure reported in several species of piscivorous birds in the Great Lakes Basin appear to be associated with excessive concentrations of bioaccumulated chemicals in various tissues (Mineau *et al.*, 1984; Price and Weseloh, 1986; Bishop and Weseloh, 1990; Gilbertson *et al.*, 1991). In the early 1970s, the concentrations of several chlorinated organic chemicals in herring gull eggs and other waterbirds from the Great Lakes were among the highest reported anywhere in the world. Results from wildlife monitoring programs in effect since 1971 indicate that the concentrations of PCBs and several other chlorinated chemicals in herring gull eggs have declined since the mid-1970s (Bishop and Weseloh, 1990) (Fig. 1).

Results reported by Newton *et al.* (1989) indicate that piscivorous birds in Britain also are recovering, with concurrent reductions in their tissue concentrations of various chemicals. Newton *et al.* (1989) conducted a detailed analysis of organochlorine and mercury concentrations in the eggs of British peregrine falcons. The authors examined possible time trends in residue levels, egg shell thickness, and analyses of chemical residues as they may relate to breeding success. The chemicals measured were DDE, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4:5,8-dimethano-

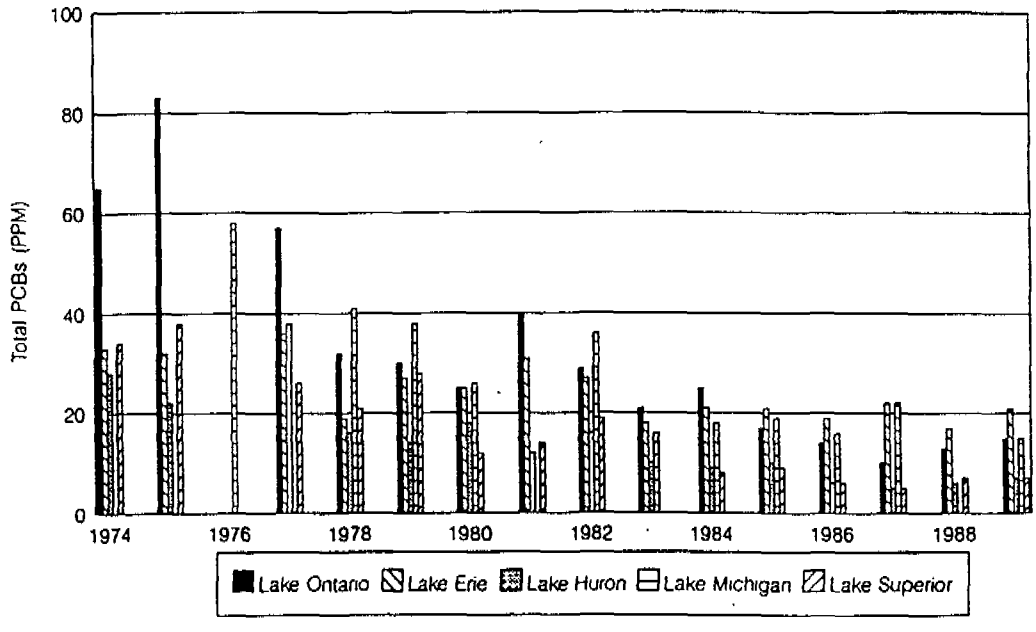

FIG. 1. Trends in annual average total PCB concentrations in herring gull eggs collected from the Great Lakes, 1974–1989. Data from Environment Canada (1991b). Snake Island, Lake Ontario; Middle Island, Lake Erie; Double Island, Lake Huron; Big Sister Island, Lake Michigan; Granite Island, Lake Superior.

naphthalene (HEOD, the active ingredient of dieldrin), PCBs, and mercury. The authors reported that the recent breeding success and recovery of peregrine falcon populations were associated with a general decline in concentrations of DDE, HEOD, and mercury in peregrine eggs over the study period, 1963 to 1986.

In summary, several studies have proposed an association between the adverse effects in wildlife populations based on field observations and various bioaccumulative chemicals, including mercury, PCBs, DDE, chlorinated dioxins, dieldrin, and some others. The weight of available evidence indicates that these chemicals, and in some cases, other causal factors including other chemicals, decreased quality of habitats, and various diseases unrelated to chemical releases are implicated in the adverse effects observed in Great Lakes piscivorous wildlife. Since the curtailment of the use of PCBs, DDT, dieldrin, and hexachlorobenzene in the early 1970s, the egg and tissue concentrations of these chemicals and by implication the exposures of wildlife to these chemicals have declined in most species around the Great Lakes (Fig. 2) (Bishop and Weseloh, 1990). Coincident with the declining tissue concentrations of chemicals, most species have exhibited some degree of population recovery, providing evidence that any adverse effects of these chemicals are reversible and appear to be dose-related.

The declining environmental concentrations of bioaccumulative chlorinated organic chemicals also are reflected in human populations (Regulatory Network Inc., 1992), although such comparisons over time may be confounded by temporal variations in the analytical procedures used and in sampling protocols. Diet is a major source of human exposure to PCBs, primarily due to the concentrations of PCBs in major components of the human diet (e.g., fish, dairy products, various red meats). Exposures of humans to PCBs through diet decreased by approximately 10-fold during the 1970s (from approximately 6.9 μg PCB/day in 1971 to approximately 0.7 μg PCB/day in 1980) and then continued to decline to 0.05 μg PCB/day by 1989, a 138-fold decrease from the concentrations in 1971 (Fig. 3).

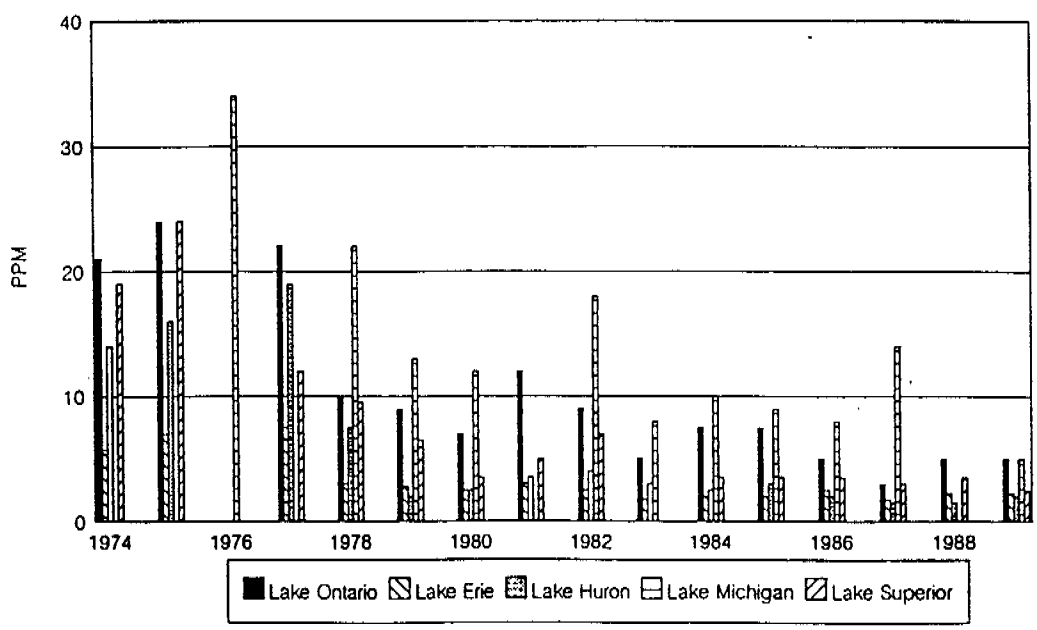

FIG. 2. Trends in annual average total DDE concentrations in herring gull eggs collected from the Great Lakes, 1974–1989. Data from Environment Canada (1991b). Snake Island, Lake Ontario; Middle Island, Lake Erie; Double Island, Lake Huron; Big Sister Island, Lake Michigan; and Granite Island, Lake Superior.

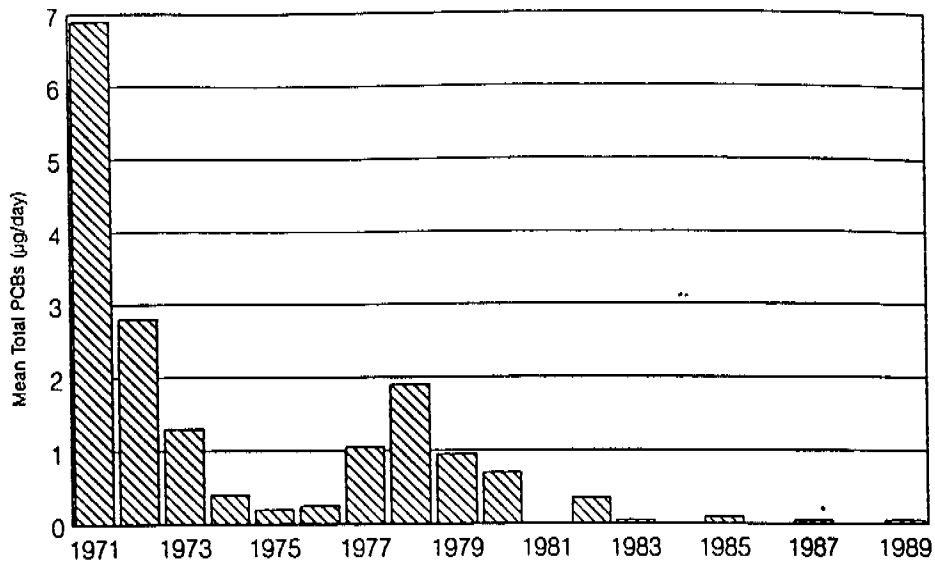

FIG. 3. FDA Total Diet Study: Adult intakes from 1971 to 1990. Data are from the Food and Drug Administration (FDA) Total Diet Study of 234 food items in the United States. 1989 represents an average of the years 1988, 1989, and 1990. Data from Regulatory Network, Inc. (1992).

Information on concentrations of PCBs in human tissue is provided from the National Human Adipose Tissue Survey (NHATS) in the United States, where adipose tissue specimens from statistically selected samples were analyzed for a series of toxic chemicals. The results were grouped as not detected, detected but less than 1 ppm, between 1 and 3 ppm, or greater than 3 ppm (Regulatory Network Inc., 1992). In 1972, 62% of the population had PCB concentrations of greater than 1 ppm compared to 2% in 1984 (Fig. 4). In 1972, 4% of the population had greater than 3 ppm of PCBs, and the proportion increased to slightly less than 10% by 1977; however, by 1983 and 1984, less than 1% of the samples had greater than 3 ppm. Robinson *et al.* (1990) reported similar trends in their independent review of the NHATS (National Human Adipose Tissue Survey) data. Results from German studies of chlorinated organic chemicals in human breast milk, although subject to differences in analytical proce-

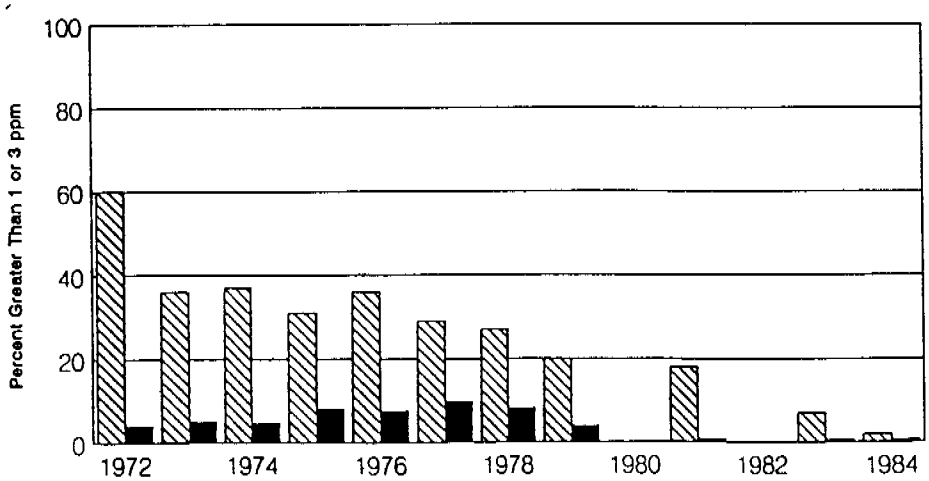

FIG. 4. National Human Adipose Tissue Survey results, 1972–1984: PCB levels greater than 1 (▨) or (■) ppm. Data from Regulatory Network, Inc. (1992).

dures, also indicate that concentrations have decreased over the past decade (1981–1991) (Beck and Heinrich-Hirsch, 1993).

Since the concentrations of chlorinated organics with greatest molecular weights have decreased in human tissues, it is reasonable to suggest that the dose also has decreased. There is no evidence that indicates conclusively that past exposure of humans to environmental concentrations of chlorinated organics has been above a threshold dose for adverse effects.

The application of the dose–response principle to exposures of wildlife and humans to certain bioaccumulative chlorinated organic chemicals demonstrates that, as chemical concentrations in the environment decline, exposures to humans and wildlife also decline. Coincidentally, in species where adverse effects were observed, these effects decrease in magnitude as exposures decrease, hindering the establishment of causal relationships between specific chemicals and the adverse effects reported, since a variety of other factors begin to play a more central role. These observations substantiate both the dose–response concept and, in cases where data are adequate, the existence of exposure thresholds below which adverse effects are not measurable. In addition, the adverse effects appear reversible as exposure diminishes, at least on a population basis.

METABOLIC CAPABILITY OF ENVIRONMENTAL SPECIES—THE SIGNIFICANCE OF NATURALLY OCCURRING CHLORINATED ORGANIC CHEMICALS

Recent information shows that more than 1500 different halogenated chemicals are produced and released into the environment by various plants, marine organisms, insects, bacteria, fungi, and various physical natural processes. The numbers of naturally occurring chlorinated organic chemicals that have been identified have expanded significantly in the past decade (e.g., 30 naturally occurring chlorinated chemicals had been identified in 1968, compared to 1500 in 1992). According to Gribble (1992), the increased interest in the area of organohalogen natural product chemistry will result in the identification of many more naturally occurring sources of organohalogen chemicals in the future. The naturally occurring chlorinated organic chemicals identified to date include chlorinated alkanes, alkenes, benzenes, phenols, PCBs, and chlorinated dioxins and furans and encompass a broad range of substances representative of the same classes of chemicals, with the same physical/chemical properties as those produced by various human activities (Gribble, 1992). The same dose–response principles govern the potential occurrence of adverse effects from these chlorinated organic chemicals, regardless of their natural or anthropogenic origin. Furthermore, the existence of natural sources of chlorinated organic chemicals demonstrates that the earth's ecosystems have been exposed to these chemicals independent of human activities and can survive normally in the presence of these types of chemicals.

Naturally occurring chlorinated organic chemicals that are released into the environment are produced by a number of organisms, including bacteria, fungi, algae, ferns, and higher plants (Siuda and DeBernardis, 1973; Fenical, 1981; Harper, 1985; Gribble, 1992; NCASI, 1992). For example, chloromethane is produced by marine algae, wood-rotting fungi, the evergreen cypress, and several other species (Gribble, 1992). Similarly, 2,4-dichlorophenol (a chlorinated aromatic) is produced by soil fungi, grasshoppers have been shown to secrete 2,5-dichlorophenol, and 2,6-dichlorophenol

is secreted as a pheromone by the female lone star tick (Gribble, 1992). In addition, many compounds that are synthesized by organisms to protect themselves from pests or competitors contain one or several chlorine atoms (Ray, 1991).

Chlorinated dioxins and furans have been produced as unwanted by-products from a variety of processes, but they are produced in significant amounts also through natural processes. They appear to be ubiquitous in the environment due to numerous chemical reactions occurring during the combustion of natural and synthetic organic materials in the presence of naturally occurring chlorine and have been detected in a wide range of particulate samples (e.g., ash samples from the combustion of untreated firewood such as birch, oak, willow, mountain ash, and applewood in fireplaces). This leads to the conclusion that natural combustion during forest fires and slash burning are contributors to the existing concentrations of these chemicals in the environment (Bumb *et al.*, 1980; Nestrick and Lamparski, 1982; Clement *et al.*, 1985; Tashiro *et al.*, 1990). An additional natural source of chlorinated dioxins and furans appears to be through the transformation of chlorophenols catalyzed by bacterial peroxidase enzyme systems, leading to their presence in contaminated sewage sludge, soils, and sediments as microbial transformation by-products from chlorophenols in sewage (Oberg *et al.*, 1991). Other natural sources of chlorinated organics include decomposition of seaweed, and volcanic activity (Travis and Hattemer-Frey, 1989; Gribble, 1992). Conversely, even though chlorinated dioxins and furans are produced by natural processes, studies of sediment cores from the Great Lakes show a correlation of increases in concentrations of these chemicals with increased human activities in the past century (Czuczwa *et al.*, 1984; Czuczwa and Hites, 1985; Hites, 1990), indicating that historical production from human activities exceeded those produced by natural processes.

While the relative contribution of natural sources of chlorinated organic chemicals to environmental concentrations would be small in areas of the greatest anthropogenic production, the relative contributions of natural sources to low-level background concentrations are considered quite significant, particularly in environments where large-scale degradation of organic material occurs (Gribble, 1992). For example, it has been estimated that 300,000 metric tons of total absorbable organohalides (AOX) are produced per year through natural processes in peat bogs in Norway (Asplund *et al.*, 1989).

The occurrence of chlorinated organic chemicals in the environment and their presence in diverse groups of mammalian and nonmammalian species demonstrate that organisms are capable of existing in the presence of certain environmental concentrations of these chemicals with no apparent adverse effects. Many chlorinated organic chemicals are metabolized by mixed function oxygenase (MFO) enzymes (Safe, 1989), an inducible, nonspecific enzyme system that metabolizes compounds into more water-soluble forms, thereby facilitating excretion (Matthews and Dedrick, 1984; Rand and Petrocelli, 1984). This enzyme system is inherent in mammals, birds, aquatic species, and some invertebrates (Environment Canada, 1991c) and is also involved in the metabolism and homeostasis of hormones and other endogenous substances. Other chemicals metabolized by this group of enzymes include caffeine (Environment Canada, 1991c), β-naphthoflavone, phytosterols found in plant tissue (Hodson *et al.*, 1992b), naphthalene, and benzo[*a*]pyrene (Rand and Petrocelli, 1984). Aflatoxin B_1, a toxin produced by fungal molds on peanuts, other nuts, and grains, also is metabolized by the MFO enzyme system (Schoenhard *et al.*, 1976). In addition to metabolizing exogenous chemicals that enter the body, MFO enzymes are responsible for the me-

tabolism of various reproductive hormones and chemicals that regulate moulting in aquatic invertebrates (Environment Canada, 1991c). Since MFO enzymes metabolize a variety of chemicals (naturally occurring and synthetic, chlorinated and nonchlorinated) by normal physiological processes, it is clear that the MFO system does not exist solely for the metabolism of chlorinated chemicals, and thus induction of MFO is not necessarily indicative of exposure to chlorine-containing chemicals.

These observations add support to the conclusion of Mackay (1992) that there is no basis for suspecting that exposures to small quantities of human-made chlorinated organics would result in the perturbation of biological systems. Furthermore, the ubiquity of chlorinated organic chemicals in the environment and the presence in diverse groups of mammalian and nonmammalian species of a common enzyme system, normally used for endogenous metabolic purposes but also capable of metabolizing chlorinated organic chemicals, provide evidence that organisms have evolved in the presence of these chemicals in the environment.

SPECIFICITY OF ASSOCIATION AND BIOLOGICAL PLAUSIBILITY OF REPORTED ADVERSE EFFECTS

There are marked differences among the various chlorinated organic chemicals with regard to potential risk from exposure. Consequently, it is important to evaluate the information carefully regarding associations between reported effects and exposures in the environment. The critical point of such an evaluation is the examination of the weight of evidence for cause–effect relationships. The establishment of reasonable cause–effect relationships based on good science is essential to invoke effective mitigation strategies. Unless this course is followed, mitigation procedures designed to resolve environmental problems may focus on the wrong factors and, consequently, be ineffective. Obtaining unequivocal or definitive proof of cause–effect relationships at a mechanistic level can be time consuming and expensive, and unwanted adverse effects could occur while waiting for "definitive proof" of causality. Therefore, a weight-of-evidence approach, combined with the application of sound scientific judgment, is needed to determine when the available evidence is sufficient to establish associations between cause and effect that are definitive enough to achieve the desired results within an environmental and socioeconomic context.

The establishment of cause–effect relationships usually is based on a combination of epidemiological or ecoepidemiological information and controlled laboratory studies. Epidemiological studies focus on the possible associations between human diseases or conditions and potential causal factors (Hill, 1965). Ecoepidemiology refers to the study of possible associations between adverse effects on components of the ecosystem (including humans) and potential causal factors (Bro-Rasumssen and Lokke, 1984). Laboratory studies to facilitate observations of cause–effect and dose–response relationships are conducted under controlled conditions where many of the variables that can affect the responses of organisms to chemicals can be controlled. Practical considerations, however, relate to the applicability of laboratory information to field or "real-world" conditions and to the relevance of field observations because of the myriad of confounding variables encountered in the real world. Hill (1965) concluded that the following features must be considered in attempting to infer causality from associations between effects and various factors.

(i) Strength of the association: How often is the event of concern observed in populations exposed to the factors of concern, compared to populations that are not exposed to these factors?

(ii) Consistency of the observed association: Has the association repeatedly been observed by different investigators, in different places, circumstances and times?

(iii) Specificity of association: Is there an association between specific populations, or to particular sites or areas and a specific disease?

(iv) Temporality of association: Is the disease or effect approximately related to the time of exposure?

(v) Is there a dose response between the disease or effect and the agent(s) involved? This has not been interpreted to mean that a statistically significant dose–response curve is required to establish causality; however, there should be reasonable evidence to enable a scientific judgment that the effect becomes greater as exposure increases.

(vi) Plausibility of association: Is the association biologically plausible based on the biological knowledge of the day? Evidence of implausibility should have more weight than actual lack of information on the biology.

(vii) Coherence of association: The cause–effect interpretation should not be seriously at conflict with the generally known facts of natural history and disease.

(viii) Is there experimental evidence supporting the association? Does removal of the associated agent cause the effect to disappear or diminish in magnitude?

(ix) Analogy of association: The effects observed are known to be caused by similar agents.

Fox (1991) grouped the essential features proposed by Hill (1965) for the inference of cause–effect relationships in ecoepidemiology studies. Four criteria considered to help to validate causal associations include:

(i) Strength of association;
(ii) Consistency of association;
(iii) Predictive performance or specificity of association;
(iv) Evidence of dose–response relationships.

Three criteria considered to indicate that various events are not causally associated include:

(i) Incompatibility on the basis of time order (e.g., the event occurs before exposure or increases as exposure decreases);
(ii) Lack of evidence of factual or biological plausibility;
(iii) Lack of consistency in replication.

If the application of sound scientific principles is to be followed, these criteria must be applied before a causal relationship is inferred between effects and exposures to specific chemicals in the environment. As indicated previously, laboratory information must be used, whenever available, to assist in interpreting associations between specific agents and effects.

Associations Based on Information from Environmental Studies

In this section, the criteria for establishing causal association, as discussed above, are applied to three reported associations between environmental factors and adverse effects:

(i) This evaluation shows that the alleged causal association between chemical exposure and effects on marine mammals is confounded by a number of factors.

(ii) In the case of potential adverse reproductive effects on fish-eating birds on the Great Lakes, the evaluation shows a relatively strong association was evident between historical exposures to certain bioaccumulative chlorinated chemicals and reproductive health, although there were likely other contributing factors. As environmental concentrations of these chemicals have decreased in the Great Lake region, the occurrence of continuing effects also has diminished.

(iii) In the case of pulp mill effluents, the evaluation shows that chlorine bleaching is not exclusively responsible for the effects reported in fish populations.

These three examples are examined in more detail in the descriptions that follow.

Adverse effects on marine mammals. Mass mortalities have occurred in marine mammals over the past decade or more in various regions, although there is considerable debate whether possible causal factors include persistent chlorinated organic chemicals or are solely expressions of natural disease processes.

Reports have been published concerning the cause of death of the common seal in the Dutch area of the Wadden Sea. Dietz *et al.* (1989) and others (Grachev *et al.*, 1989; Harwood *et al.*, 1989; Osterhaus *et al.*, 1989) reported that the cause of death for these seals was most likely a virus resembling canine distemper virus. Furthermore, these authors reported a number of viruses associated with mass mortalities of the seals, including influenza, herpes, and canine distemper virus. These viruses are spread in aerosols when infected seals cough. The mass mortality of Lake Baikal seals in 1987, of New England harbor seals in 1979–1980, and of crabeater seals in the Antarctic in 1955 were also attributed to viral infections (Geraci *et al.*, 1982, 1984; Hinshaw *et al.*, 1984; Grachev *et al.*, 1989). Furthermore, Geraci *et al.* (1982) reported that most affected populations were showing increases in numbers prior to the outbreak of the disease, which suggests a relatively healthy population or one showing no overt signs of adverse effects. Thus, it is unclear how cumulative chemicals could be causally related to such short-term events. The New England harbor seal population had doubled since 1972 at the time of the disease outbreak, in 1979–1980. Similarly, the crabeater seal population was greater than normal in 1955, when 85% of the population died of a viral pneumonia infection. Interestingly, milder winter temperatures occurred during both these epidemics, resulting in seals spending more time out of water and congregating on land (Geraci *et al.*, 1982). This closer contact would facilitate the spread of the diseases. These facts implicate a causative factor other than environmental concentrations of persistent chlorinated organic chemicals since tissue concentrations and environmental concentrations of such chemicals change only slowly with time.

It has been suggested that viral epidemics may be a result of organochlorine-induced impairment of the immune system (Brouwer *et al.*, 1989). Effects on the immune system have been observed in laboratory animals following exposure to DDT (Rehana and Rao, 1992), toxaphene (Allen *et al.*, 1983), dieldrin (Exon *et al.*, 1987), lindane (Allen *et al.*, 1979), hexachlorobenzene (HCB) (Allen *et al.*, 1979), chlorinated dioxins and furans (Silkworth and Vecchi, 1985), PCBs (Silkworth and Vecchi, 1985), and polybrominated biphenyls (PBBs) (Thomas and Faith, 1985). Exposure of laboratory animals to extreme doses of these chemicals causes suppression of antibody responses, atrophy of lymphoid organs, and increased susceptibility and sensitivity to toxic or infectious agents. Other chemicals that have been demonstrated to cause immunological

effects, also at extreme doses relative to usual environmental exposures, are polycyclic aromatic hydrocarbons, benzene, lead, inorganic mercury, and other metals (Dean and Murray, 1991). Consequently, wild animals could be exposed to several chemicals that affect the immune system.

It has also been suggested that a mechanism possibly explaining PCB-induced immune effects allegedly leading to disease in the common seal may be related to deficiencies in vitamin A and thyroid hormones (Brouwer *et al.*, 1989). Vitamin A (retinol in its various forms) is a fat-soluble vitamin, important for normal vision, reproduction, and development in marine mammals (Environment Canada, 1991c). Thyroxine is a thyroid hormone which is important for normal growth and development. Seals fed fish from the Wadden Sea, having significantly greater concentrations of DDE and PCBs than fish from the northeast Atlantic, had lesser concentrations of plasma retinol and thyroxine. Since laboratory studies with experimental animals have indicated an effect of PCBs in regulation of vitamin A and thyroid hormones, Brouwer *et al.* (1989) suggested that the PCBs with non-*ortho*-chlorine substitutions, or hydroxy metabolites of non-*ortho*-substituted PCBs, impair normal vitamin A and thyroxine homeostasis, which in turn causes reduced concentrations of vitamin A and an increased susceptibility to stress. It has been suggested that reduction of both retinol and thyroxine may be caused by the interference of PCBs, or metabolites of PCBs, or of secondary products related to effects caused by these chemicals, with the retinol–thyroxine plasma carrier–protein complex (Brouwer *et al.*, 1989). The reduced levels of vitamin A and immunoreactive thyroid hormones have been suggested to result in reduced reproductive success, retarded growth, and increased susceptibility to infection (Reijnders, 1986; Brouwer *et al.*, 1989; Manson and Wise, 1991; Schumacher *et al.*, 1991). Bergman and Olsson (1985) also speculated that PCBs in the diet and other chlorinated organic chemicals have caused adverse effects on the endocrine system of Baltic seals, leading to immunosuppression and interrupted pregnancies. The results from these studies are difficult to interpret since, although effects observed in the Baltic seals may be the result of excessive exposure to PCBs, the existence of viral epidemics in relatively pristine environments and the fact that the Baltic Sea is contaminated with numerous other chemicals that affect the reproductive and immune systems seriously confound the identification of a single causal factor.

Similarly, some attempts have been made to link the mass mortality of bottlenose dolphins along the U.S. Atlantic coast (1987–1988) to organochlorine contamination associated with compromised immune function. However, the results of a comprehensive investigation concluded that the dolphin mortalities were due to contamination with excessive levels of a natural neurotoxin produced by dinoflagellate algae (Geraci, 1989). Blooms of these algae, also known as "red tide," are known to occur on the east coast, but are particularly common on the Gulf Coast of Florida. During the summer and fall of 1987, blooms were carried north by the Gulf Stream along the east coast resulting in human poisoning from the consumption of affected fish (Bonaventura and Bonaventura, 1987) and the temporary closure of the shellfish industry (Tester *et al.*, 1989). Geraci (1989) questioned the significance of environmental contaminants in the resilience of these animals to infection, and it was concluded that the mass mortalities of bottlenose dolphins observed on the east coast of the United States were due to a natural plant toxin (Geraci, 1989). Therefore, the weight of evidence indicates that the occurrence of mass mortalities in marine mammals is likely due to

natural disease factors and the effects of weather and factors related to habitat. It is doubtful that there is a causal relationship with chlorinated organic chemicals.

Adverse reproductive effects on birds. A second example of the inference of cause–effect relationships involves environmental concentrations of chlorinated organic chemicals and a variety of effects observed in piscivorous birds. Based on the interpretation of the results from laboratory and field studies, a causal association has been inferred in Great Lakes piscivorous birds between highly chlorinated organic chemicals and a disease known as chick edema, a syndrome characterized by embryo mortality, edema, and deformities (referred to as "GLEMEDS" by the authors) (Gilbertson *et al.*, 1991). An increased incidence of abnormalities, including bill defects and eye and foot deformities, was observed in colonies of common and roseate terns at Long Island Sound in 1969 and 1970 (Hays and Risebrough, 1972). Eggs from these colonies were found to contain greater concentrations of PCBs than those from reference colonies. Gilbertson (1983) reported high mortality, edema, porphyria, liver enlargement, fatty infiltration, and necrosis, as well as growth retardation and embryo deformities (symptoms similar to chick edema disease) in Great Lakes herring gulls. Retrospective analyses of the eggs revealed the presence of significant concentrations of the chick-edema active compounds (chlorinated dioxins and furans) and coplanar PCBs (Stalling *et al.*, 1985; Kubiak *et al.*, 1989). Concentrations of PCBs in eggs of Forster's tern associated with adverse reproductive effects (reduced hatchability, delayed hatch, reduced number of fledglings) were: total coplanar PCBs, 1.37 to 41 ppb (wet weight (ww); total PCBs, 6.2 to 25.9 ppm (ww) (Kubiak *et al.*, 1989).

Yamashita *et al.* (1993) suggested a role for chlorinated organic pesticides and PCBs in the reproductive disorders observed in double-crested cormorant and Caspian tern chicks from the upper Great Lakes (Kurita *et al.*, 1987; Kubiak *et al.*, 1989). Eggs from these species contained concentrations of PCBs and DDE in the range where reproductive effects had been observed in Forster's tern and British peregrine falcon. Of all chemicals analyzed, concentrations of DDE and PCBs occurred at the greatest concentrations. Concentrations of PCBs ranged from 3.6 to 14 ppm (ww) while concentrations of DDE ranged from 2.2 to 6.3 ppm (ww). These PCB concentrations were in the lower range of concentrations needed for adverse reproductive effects on Forster's tern reported by Kubiak and coworkers (1989). DDE concentrations were in the range of the threshold concentration for adverse effects to peregrine falcon (3 ppm) reported by Newton *et al.* (1989).

In considering the criteria for causality as elaborated by Hill (1965) and Fox (1991) with respect to an association between PCBs and DDE and effects on bird reproduction the following points may be made: (i) laboratory studies affirm the plausibility of an association, and environmental concentrations were relevant to the effects observed; (ii) changes in environmental concentrations over time and coincident changes in response reinforce a possible dose–response and temporal relationship; and (iii) consistency of association is evident in several studies on different species and in different areas. In the absence of information that could negate the association (as per Fox, 1991), the weight of evidence appears to indicate that the reported adverse effects on the reproductive health of fish-eating birds were likely associated with exposures to PCBs and DDE; however, since wild animals are exposed to other bioaccumulative agents that may also affect reproductive health, it cannot be concluded definitively that PCBs and DDE are the sole causal factors.

The possibility that other factors may influence reproduction is evident from the results of controlled laboratory studies as well as from biomonitoring data. For example, in laboratory studies, fish exposed to PCBs at concentrations similar to those found in the environment had reduced survival, reproduction, and growth (Mayer *et al.*, 1985). However, mortality of feral chinook salmon eggs reared in a laboratory was not correlated to PCB egg residues (Williams and Giesy, 1992). In addition, mortality of Lake Michigan lake trout fry increased from 22 to 92% between 1975–1976 (Berlin *et al.*, 1981) and 1980–1981 (Mac *et al.*, 1985), while the total environmental concentrations of PCBs and DDT decreased over that time frame. Williams and Giesy (1992) concluded that the lack of correlation between various PCB exposure parameters and survival of eggs and fry may indicate that other factors affecting habitat such as large amounts of suspended sediments, excessive quantities of sewage discharge, untreated sewage, and elevated ammonia concentrations were involved in the rearing mortality observed.

Pulp mill effluents. A third example of possible cause and effects based on field observations involves effects noted in fish exposed to pulp mill effluents (Södergren *et al.*, 1988; Södergren, 1989). Pulp mill effluent has received a great deal of attention concerning toxicity and the presence of chlorinated organic chemicals, particularly dioxins and furans. Pulp mill effluents contain complex chemical mixtures, and the toxicity of the effluents varies, depending on the pulping and bleaching technology employed, the treatment system used, and even the species of tree used to make the pulp (Salkinoja-Salanen *et al.*, 1984; McLeay and Associates, 1987). A great deal of research has been directed toward identifying the constituent(s) in mill effluent responsible for the effects noted in fish populations.

It has been suggested that the toxicity of pulp mill effluents may be regulated using the measure of total adsorbable organic halide (AOX), representing the total concentration of all chlorinated and other halogenated materials of all types in the effluent. However, the relative chemical composition of AOX (hence, potential toxicity) in effluent varies considerably, both between mills and temporarily within a specific mill with respect to the proportions of high and low molecular weight substances. Since toxicity is directly related to exposures arising from the relative concentrations of specific chemical constituents in the receiving waters in a dose-dependent manner, AOX alone is not adequate to describe the potential toxicity of the pulp mill effluent.

A variety of nonlethal endpoints ranging from effects on growth and development to biochemical and hormonal changes have been associated with the exposure of aquatic species to pulp mill effluent. These effects must be examined critically to ascertain what constituent(s) is specifically associated with and, subsequently, what process should be controlled to minimize adverse environmental effects.

Increased liver enzyme activity of mixed function oxygenases (MFO) has been used as an indicator of exposure of wild fish to aromatic hydrocarbons, although such enzyme changes do not in themselves indicate the occurrence of adverse effects (Payne *et al.*, 1987; Environment Canada, 1991d). Induction of MFO activity has been observed in fish collected downstream of pulp mill outfalls in Canada (Rogers *et al.*, 1989; Smith and Rokosh, 1989; Munkittrick *et al.*, 1989, 1991; Servos *et al.*, 1992; Hodson *et al.*, 1992a) and Scandinavia (Larsson *et al.*, 1988; Södergren *et al.*, 1988; Andersson *et al.*, 1988; Lindstrom-Seppa and Oikari, 1989, 1990a,b), compared to reference fish not exposed to pulp mill effluent. It has been suggested that increased MFO activity may lead to enhanced metabolism of circulating concentrations of sex

hormones thereby causing reproductive effects that have been noted in fish populations (McMaster *et al.*, 1991; Munkittrick *et al.*, 1991). Although the substance(s) responsible for the observed MFO induction near pulp mills has not been identified (Munkittrick *et al.*, 1992a; Hewitt *et al.*, 1992), chlorinated dioxins and furans have been proposed as causative agents (Rogers *et al.*, 1989; Hodson *et al.*, 1992a; Lockhart and Metner, 1992).

More recent information, however, suggests that constituents in bleached kraft mill effluent other than chlorinated dioxins and furans may be responsible for increased MFO activity in fish (Munkittrick *et al.*, 1992a,b). Enzyme induction by chlorinated dioxins and furans has been shown to persist for 85–180 days under laboratory conditions (Muir *et al.*, 1990; Van der Weiden *et al.*, 1990). However, MFO activity in three species of fish, collected downstream of secondary treated pulp mill outfall, returned to control values following a planned mill maintenance shutdown and a 2-week effluent-free exposure period (Munkittrick *et al.*, 1992a). These observations refute the contention that chlorinated dioxins and furans, or other such chlorinated organics, are a major cause of MFO induction by pulp mill effluent.

Moreover, data presented by Van der Kraak *et al.* (1992) and Martel and Kovacs (1992) also suggest a causative factor for MFO induction other than chlorinated dioxins and furans or other chlorinated organic chemicals. These studies have shown that enzyme induction and changes in other biomarkers such as increased liver size and decreased gonad size, often used as indicators of adverse effects, are evident in fish populations in waters receiving effluents of pulp mills that do not use chlorine in their bleaching process (Martel and Kovacs, 1992). In fact, the most recent observations of Andersson (1992) show that significant reductions in the use of chlorine between 1988 and 1990 at the Norrsundet pulp and paper mill on the Baltic Sea did not result in corresponding reductions in the biomarkers of increased liver size, blood lactate, or MFO enzyme activity in exposed fish. Thus, increasing evidence suggests that something other than high molecular weight chlorinated organic chemicals may be largely responsible for the effects noted in fish exposed to pulp mill effluents.

Possible etiological agents may be natural plant sterols (phytosterols). Phytosterols were implicated as causal factors in the masculinized female mosquitofish observed in a northwest Florida stream receiving pulp and paper mill effluent (type not specified) (Howell *et al.*, 1980). Female mosquitofish possessed male-like gonopodial anal fins and demonstrated elements of male reproductive behavior. Similar observations were made for three fish species (least killifish, sailfin molly, and mosquitofish) in another Florida stream receiving pulp and paper mill effluent (type not specified) (Howell *et al.*, 1980; Bortone and Drysdale, 1981). The acquisition of male secondary sex characteristics among poecilid female fish was observed and was reproducible in the laboratory using exposure to microbial-degraded phytosterols. This process is considered analogous to processes occurring in effluents from kraft pulp mills. Although the masculinized females were still able to produce viable offspring, preliminary laboratory studies demonstrated that their reproductive fitness may be impaired compared to controls (Davis, 1989). Thus, the masculinization of female fish inhabiting areas receiving pulp mill effluent appears to have resulted from exposure to natural plant sterols rather than process-related chlorinated organic chemicals.

These studies of field populations, particularly of migratory marine mammals, are difficult to interpret. Unlike laboratory experiments in which any factor that may influence the results is controlled, confounding factors abound in field studies making

it difficult to distinguish cause–effect relationships. For example, the evidence shows confounding effects of multiple chemicals, interaction with habitat, and various natural disease processes. In the pulp bleaching example, the lack of consistency in observation between chlorine bleaching and adverse effects also confound the interpretation of causal relationships. Therefore interpretation of results based on field studies should be made with caution, bearing in mind that unknown factors may influence the results. Consequently, cause–effect relationships are difficult to demonstrate and the specificity of association may be weak. The three examples presented serve to demonstrate the necessity of using the criteria for assessing causal associations (Hill, 1965; Fox, 1991) between chemicals and reported effects on organisms in the environment.

Associations Based on Information from Human Studies

Examples of difficulties similar to those outlined above for wildlife also are seen in attempting to establish cause–effect relationships according to the criteria outlined by Hill (1965) and Fox (1991) based on information from human populations. The available studies often are difficult to interpret due to confounding factors such as alcohol consumption, tobacco smoking, nonprescription drug consumption, a variety of dietary differences, and exposures to a mixture of different chemicals.

Breast cancer and chlorinated organic chemicals. A causal association between several chlorinated organic chemicals (e.g., PCB, DDT, and chlorinated dioxin/furans) and the incidence of human breast cancer has been proposed (Falck *et al.*, 1992). The associations between breast cancers and breast tissue concentrations of bioaccumulative chlorinated organic chemicals proposed by Falck *et al.* (1992) does not meet the requirements for causality as outlined by Hill (1965) and Fox (1991). First, the proposed association does not appear to be biologically plausible based on information from laboratory studies where doses and confounding factors are more readily controlled. Laboratory studies on chlorinated dioxins/furans, PCBs, and DDT have been shown to increase liver tumors but not mammary tumors. The available evidence demonstrates that the increased incidence of liver tumors observed in these animal studies is related to the promotion of preexisting spontaneous lesions (Maslansky and Williams, 1981; Ito *et al.*, 1982; Hayes, 1982; Poland, 1984; Safe, 1989; Silberhorn *et al.*, 1990). In fact, decreases in tumors of the mammary gland and various reproductive organs, as well as liver tumors at the lowest dose, were observed in some animal studies (Kociba *et al.*, 1978). The evidence from the experimental animal data argues strongly against the biological plausibility of any proposed relationship between exposure to these chlorinated organic compounds and breast cancer (Kimbrough *et al.*, 1975; Rossi *et al.*, 1977; Kociba *et al.*, 1978; Cabral *et al.*, 1982).

Second, the causal association proposed by Falck *et al.* (1992) does not meet the requirement of observational consistency. Several publications have reported increased concentrations of bioaccumulative chlorinated organic chemicals in human tissues (Hoffman *et al.*, 1967; Radomski *et al.*, 1968; Casarett *et al.*, 1968; Unger and Olsen, 1980; Unger *et al.*, 1982, 1984; Teufel *et al.*, 1991); however, there is no consistency among the various studies in the association of the increased tissue concentrations and specific human diseases. Inconsistency or lack of replication in observations between studies has been identified as a major factor in detracting from cause–effect relationships based on epidemiological or ecoepidemiological information (Fox, 1991).

Third, the criteria for evidence of dose–response relationships required to establish causality of association (Hill, 1965; Fox, 1991) are not consistently met in proposing a causal association between human breast cancer and concentrations of persistent chlorinated organic chemicals in breast tissue. The differences in tissue concentrations of PCBs and DDE between women with breast cancer compared to those with benign breast disease were small, within the analytical error of the method, and within the range of concentrations observed in the general population. In addition, a number of factors known to affect breast cancer (e.g., smoking habits, age, diet, and family history of breast cancer) were not controlled in the study. In fact, the women with breast cancer averaged 8 years older than those with benign breast disease. This is an important variable since (i) the incidence of breast cancer is positively correlated with age, and (ii) the concentrations of PCBs and DDE in the tissues of humans are known to increase with age regardless of breast cancer status (Kreiss *et al.*, 1981).

Based on the above analysis, it is evident that the proposed causal association of breast cancer and exposures to bioaccumulative chlorinated organic chemicals should be rejected because of (i) lack of biological plausibility based on no increased mammary tumor incidence in laboratory studies involving PCBs and DDT, (ii) lack of consistency in observation, and (iii) lack of evidence of a dose–response relationship. In addition, the lack of information on several factors known to affect breast cancer incidence (e.g., hormonal imbalance, age of first pregnancy, evidence of miscarriage, length of nursing, age of menarche, and age at menopause) weaken the strength and consistency of association. These factors, combined with the weak nature of most of the reported associations between chemical exposure and breast cancer, lead to the conclusion that the available epidemiological data do not support a causal association between exposure to chlorinated organic chemicals and increased breast cancer incidence.

Human development and PCB exposure. The basic criteria for judging the causality of associations (Hill, 1965; Fox, 1991) also are not met by the studies proposing causal associations between human development and exposures to PCBs. Lower birth weights and smaller head circumference have been reported for infants born to mothers consuming greater amounts of fish from the Great Lakes than control (low fish consuming) populations (Fein *et al.*, 1984). The strength of the proposed association between exposures to PCBs and the observed developmental effects is seriously weakened by several factors. First, the authors suggested that PCBs in the fish were the cause of these effects, although no correlation was observed between fish consumption and umbilical cord blood PCB concentrations (IDSP, 1987; Paneth, 1991). Also, there are a number of inconsistencies in the reported analyses for PCBs in maternal and cord blood sera and in breast milk. It appears that analyses for PCBs were not conducted on serum samples with lipid concentrations less than 200 mg/dl, a decision that could bias the results in an undetermined manner. Mean maternal serum concentrations of Aroclor 1260 were somewhat greater than 9 ppb in the group consuming 52 to 183 meals of fish per year, within the normal range observed in most U.S. populations in the early 1980s. The deficiency in the criteria for a dose–response relationship (e.g., the lack of consistent correlation between cord blood PCB concentrations and fish consumption), a basic requirement for establishing causality (Hill, 1965; Fox, 1991), and the lack of substantial differences between the concentrations of PCBs in the study and the general population indicate that the effects observed on human development in the populations studied were not causally related to exposures to PCBs.

Second, the women from the elevated fish consumption group also reported significantly greater consumptions of alcohol, caffeine, tobacco, and cold remedy medications during pregnancy than those consuming less fish, and the statistical evaluation of these confounding factors was not appropriate (Environment Canada, 1991a; Paneth, 1991). Many of these factors are known to result in lower birth weights of infants and lower developmental scores. Reduced head circumference and weight were not correlated with cord serum PCB levels when considering potential confounding effects of alcohol (a known teratogen), caffeine, and drug use during pregnancy. In addition, there were differences in maternal body weights (the high-fish consumers weighed on average 4.1 kg less) prior to pregnancy, and prepregnancy body weight is one of the major factors influencing infant birth weights. Furthermore, of the population consuming greater quantities of fish, the proportion with nonspontaneous deliveries was almost 50% higher than in the low fish-consuming group, and infants born following nonspontaneous deliveries are more prone to apparent developmental deficits as newborns (Paneth, 1991). Other factors that were not considered in the assessment were the concentrations of other chemicals known to be present in fish from the Great Lakes. All these confounding factors seriously detract from the biological plausibility of an association between the amount of exposure to PCBs, as indicated by blood PCB concentrations, and effects on human development.

Third, the plausibility and consistency of association that are required to establish a causal relationship (Hill, 1965; Fox, 1991) are also seriously weakened by various follow-up studies of the initial pregnancies assessed by Fein *et al.* (1984). Greater umbilical cord serum concentrations of PCBs were marginally associated with poorer verbal and memory scores on the McCarthy Scales performance tests and with lower scores in the verbal and numerical memory subtests in the same children 4 years later (Jacobson *et al.*, 1990a). Other components of the McCarthy Scales performance tests (perceptual performance, quantitative, motor, and general cognitive index) were unrelated to *in utero* exposure to PCBs. Since no correlation was observed between fish consumption and umbilical cord concentrations of PCBs, however, no correlation can be made between contaminated fish and scores on the McCarthy Scales performance tests.

The arguments for plausibility of association are also weakened by observations that, even though postnatal lactational exposures to PCBs are far greater than those reported *in utero* (Jacobson *et al.*, 1989), the children exposed to PCBs via lactation for longer periods had significantly higher scores on both the Memory and Verbal Scales tests (Jacobson *et al.*, 1990a). Poorer scores were significantly associated only with the highest maternal milk concentrations of PCBs (1250 to 2600 ng PCB/ml milk). Furthermore, the results of the Jacobson *et al.* (1990a) study also indicated that in the 4-year-old children higher serum PCB concentrations were not associated with any cognitive deficits. The plausibility of the association proposed by Jacobson *et al.* (1990a) is further eroded by the observation that certain marginal deficits reported in some clusters of the McCarthy tests were associated with higher serum concentrations of PCBs in the mother, but not with greater exposures to PCBs through lactation. Furthermore, it was not apparent that the scores obtained in the tests of any of the children were outside the ranges of normal since no such ranges were given. Finally, a total of approximately 38 behavioral and neurological tests were conducted on the children, even though the results of only two tests were reported to be affected. Some association based on chance alone would be expected from this large number of tests.

In addition, the assessment of the test results was based on a "clustering" approach, which is not a standard procedure in evaluating neurological tests from children. No information was available on the effect of the clustering on the interpretation of the results that would be expected from the general population. Therefore, it is not possible to evaluate the importance of this approach in the interpretation of the results of the studies.

The plausibility of the association proposed by Fein *et al.* (1984) is further degraded by inconsistencies in the information reported in different publications of the studies. Jacobson *et al.* (1990b) reported, based on Fein *et al.* (1984), that prenatal exposure to PCBs through mothers consuming fish was associated with a lower birth weight. The same children, assessed 4 years later, had the greatest serum concentrations of PCBs (mainly as a result of lactational exposure) and were also reported by Jacobson *et al.* (1990b) to have reduced activity levels. This result, however, is not in accordance with findings of Jacobson *et al.* (1990a) that the children who had longer lactational exposure had no cognitive deficits when compared to children with shorter lactational exposure. In fact, prior to the controlling of confounding variables, the children with longer lactational exposure tended to score higher on the McCarthy Scales performance tests (Jacobson *et al.*, 1990a). To explain this finding in the first follow-up study, Jacobson *et al.* (1990a) suggested that the children who were breast-fed longer had the greater intellectual stimulation from their mothers. If this argument is accepted, it is difficult to explain "reduced activity levels" in those 4-year-old children having relatively higher serum PCB concentrations since most of these children were part of the group of infants breast-fed for longer periods of time. In addition, no ranges of normal were included for any of the data sets from either the first or second follow-up study.

Fourth, the criterion of consistency of observation, that is an essential component in the establishment of causality of association based on epidemiological data (Hill, 1965; Fox, 1991), is not met by the studies reported by Fein *et al.* (1984) and Jacobson (1990a,b). In contrast to the results reported by these researchers, exposure to PCB (as measured by maternal serum PCB concentrations) was correlated with increased birth weight, not decreased birth weight, in a random sample of 100 participants of a 1112 women study in the Green Bay area of Wisconsin (Dar *et al.*, 1992) (near the same area where numerous effects were observed in wildlife as discussed in the previous section). Although the concentrations of PCBs reported in the maternal serum in the Dar *et al.* (1992) study would appear to be lower than those observed by Fein *et al.* (1984), the analyses conducted by Dar *et al.* (1992) were congener specific rather than for total concentrations of PCBs. Greater birth weights, as opposed to lesser birth weights, as reported by Fein *et al.* (1984) and Jacobson *et al.* (1990b), have also been reported in other fish-consuming populations (Olsen *et al.*, 1990). Similarly, Fitzgerald *et al.* (1992) observed that the concentrations of PCBs in breast milk were the same (although the congener profiles were different) between 53 Mohawk women consuming fish from the St. Lawrence River and a population of 109 women from Warren Schoharie County in New York. The Mohawk women were expected to have higher PCB concentration in their breast milk as they were determined to consume considerably more fish containing PCB prior to pregnancy (more than 1 year) than those women residing in Warren Schoharie County. These observations demonstrate a lack of consistency between fish consumption, concentrations of PCBs in breast

milk and serum, and reproductive outcomes compared to the report by Fein *et al.* (1984).

Studies designed to evaluate possible causal associations between human development and exposures to PCBs, DDT, and DDE using a cohort of children from North Carolina were conducted by Rogan *et al.* (1986a,b). Prenatal exposures of infants to PCBs, DDT, and DDE were predicted from extrapolations across the nursing period based on breast milk analysis of one sample at one stage of nursing. Calculated daily chemical exposures of the infants were based on these predicted concentrations of the chemicals in breast milk, and no validation of these predictions was reported. The neurological development of the infants was then correlated with breast milk fat to estimate a no-observable-adverse-effect level (NOAEL) of 3.4 and 1.0 ppm, respectively for Brazelton and Baileys scores (Tilson *et al.*, 1990). The Brazelton scores were based on 27 behavioral tests and 20 reflex tests that were summarized into clusters (Rogan *et al.*, 1986b) following the procedures outlined by Jacobson *et al.* (1984). Only the cluster scores for tonicity and reflexes were correlated with exposures to PCBs or DDE. Although effects on certain of the various behavioral parameters were correlated with estimated PCB exposures, the plausibility of the results, and therefore the strength of the association, is weakened by the fact that the women with higher milk PCB concentrations were older, had a greater rate of alcohol consumption, and greater tobacco smoking incidence. All of these factors are known to affect scores on a wide range of neurological testing regimes for newborn infants. In addition, the effects were observed on a small number of infants (49 infants of a total of 856 were exposed to milk concentrations of PCBs above 3.4 ppm, which is reported as the NOAEL, and only 42% of these, or 21/856, showed positive tests). The small sample size of infants exposed to PCB concentrations in breast milk fat in excess of 3.4 ppm, and who scored significantly lower on the behavioral tests, precludes the determination of a NOAEL with a high degree of confidence, for effects on behavioral testing parameters. As a result of the small sample size of infants exposed to PCB concentrations of greater than 3.4 ppm in breast milk fat, and due to the uncertainties introduced into the analysis arising from the presence of confounding variables, there is little basis for establishing a causal association between the observed effects and exposure to PCBs.

Based on the above analysis, and considering the marginal significance of the observations (most at or just below $P < 0.05$), the information reported by Fein *et al.* (1984), Rogan *et al.* (1986a,b), and Jacobson *et al.* (1990a,b) does not meet the criteria as outlined by Hill (1965) and Fox (1991) for the establishment of a causal association for an effect of PCBs on growth and behavior in human populations.

CONCLUSIONS REGARDING THE APPLICATION OF SCIENTIFIC PRINCIPLES

In this brief review, certain scientific principles have been presented that must be considered in any critical evaluation of literature reports of adverse effects of chlorinated organic chemicals. These key principles represent essential criteria by which reports should be examined, either to enable validation of the findings based on scientific principles or to show that the findings do not withstand objective scientific scrutiny. These scientific principles, as they apply specifically to the potential effects of chlorinated organic chemicals in the environment, are:

(1) the fate and biological activity of a compound are determined by the chemical properties of the compound;

(2) compounds do not show adverse effects below certain threshold concentrations, and the magnitude of response is related to dose;

(3) inherent metabolic processes allow organisms to accommodate low doses of chlorinated organic chemicals;

(4) observations associated with the presence of certain compounds must be biologically plausible effects, based on the specificity of the compound's activity in experimental systems.

This review has presented a critical evaluation of evidence relating specifically to chlorinated organic compounds by using these scientific principles. The evidence shows that different types of chlorinated organic compounds have very different types of environmental behavior and biological activity, depending upon their physical/chemical properties. These differences suggest logical ways for controlling exposure to prevent or minimize the potential for adverse effects. For example, chemicals with a greater degree of electrophilicity, such as vinyl chloride monomer, will not bioaccumulate in environmental species, but exposed workers were historically at risk (Doll, 1988). This emphasizes the importance of a containment procedure that prevents exposure of workers and the general public to the unreacted compound. On the other hand, the high lipophilicity and only gradual breakdown of certain highly chlorinated compounds point out the potential for their bioaccumulation in food chains. Problems that have been observed in certain wildlife species, notably piscivorous birds, can be associated with elevated concentrations of certain of these bioaccumulative compounds. Such unwanted effects indicate that, in the past, environmental releases of compounds with these properties were too great. Furthermore, environmental recoveries have been noted to be associated with lower environmental concentrations of such chemicals, except in localized hot spots. This dose–response evidence indicates a practical environmental threshold, even for highly bioaccumulative compounds. Scientists now know the physical/chemical properties of chemicals that cause such undesired characteristics, and the future production of such chemicals can now be avoided.

These types of scientific evidence should govern industrial design and regulatory decision making, with the goal of preventing potential adverse effects on the environment. For example, all factors that influence the rate of environmental loading of bioaccumulative compounds should be considered. Mitigating practices that would have an impact on environmental releases of chemicals include recovery and recycling to minimize the need for disposal, with the best available technology to be used for treating waste that contains compounds that could accumulate in the environment. The physical and chemical properties determine what practices would be required to ensure that environmental release rates of specific chemicals are maintained below those that would result in adverse effects. This information, coupled with scientific evidence on dose–response relationships, provides the guidance necessary to establish no-effect levels in the receiving environment that in turn will determine the rigor of waste reduction and waste treatment practices.

It is also essential to note that alternatives to any chemical product or process must be evaluated with the same scientific principles to predict potential impact on human and environmental health. The chemical characteristics of chlorobenzyltoluenes, as

substitutes for PCBs, for example, indicate that they may have similar biological characteristics (Mark *et al.*, 1990) and environmental fate (Wester and van der Valk, 1990) as PCBs. Thus, their potential quantity and rate of release into the environment should be consistent with levels that would not lead to adverse effects through their bioaccumulation. In addition, although DDT has been associated with adverse effects in wildlife species, its acute toxicity in humans and other nontarget mammals is much lower than many of the compounds introduced to take its place. Accidental poisonings and deaths from lindane following its increased use as a replacement for DDT, and from organophosphorus insecticides that more generally have replaced the organochlorine insecticides, frequently have been observed in humans and wildlife. Scientific evaluation of alternatives may lead to important decisions not only about the overall effect of the alternatives, but also about use patterns of the original product. For example, the use of DDT may be much more important in public health for the control of malaria than its past use in agriculture.

In conclusion, chlorinated organic compounds represent diverse groups of chemicals, a limited number of which can bioaccumulate in the environment. Depending on environmental release rates, concentrations of these bioaccumulative chemicals could result in exposures sufficiently great as to be associated with adverse effects in wildlife. These types of chemicals require careful consideration and control. However, evaluation according to the guiding scientific principles indicates that the vast majority of chemicals produced from chlorine chemistry do not have these chemical characteristics. Rather, they are subject to degradation in the environment and there is no reason to speculate that they would accumulate to concentrations that would pose a threat to ecosystem health. The analysis of dose–response relationships demonstrates that exposures from the environmental concentrations of nonbioaccumulative chlorinated organic chemicals are far below those that would be associated with adverse effects based on laboratory studies. There is evidence that all major classes of chlorinated organic chemicals produced by human activities also are produced by natural processes. The significance of the quantities of chlorinated chemicals produced by natural processes is not well understood, but their existence clearly demonstrates that the earth's ecosystems have evolved with a metabolic capacity to recognize and accommodate the presence of such chemicals.

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REFERENCES

Agriculture Canada (1987). *Pentachlorophenol: Wood Preservative.* Discussion document. Pesticides Directorate, Agriculture Canada. Ottawa, Ontario. 87-02.

ALLEN, J. R., HARGRAVES, W. A., HSIA, M. T. S., AND LIN, F. S. (1979). Comparative toxicology of chlorinated compounds on mammalian species. *Pharmacol. Ther.* 7, 513–547.

ALLEN, A. L., KOLLER, L. D., AND POLLOCK, G. A. (1983). Effect of toxaphene exposure on immune responses in mice. *J. Toxicol. Environ. Health* **11**, 61–69.

ANDERSSON, T., FORLIN, L., HARDIG, J., AND LARSSON, A. (1988). Physiological disturbances in fish living in coastal waters polluted with bleached kraft mill effluents. *Can. J. Fish Aquat. Sci.* 45(8), 1525–1536.

ANDERSSON, T. (1992). *In Vivo and In Vitro Methods to Assess the Toxicity of Pulp and Paper Mill Effluents to Fish.* Presented as the 7th Colloquium on Pulp and Paper Mill Effluents, December 15 and 16, 1992. Pulp and Paper Centre, University of Toronto, Toronto, Ontario, Canada.

ANKLEY, G. T., LODGE, K., CALL, D. J., BALCER, M. D., BROOKE, L. T., COOK, P. M., KREIS, R. G., CARLSON, A. R., JOHNSON, R. D., NIEMI, G. J., HOKE, R. A., WEST, C. W., GIESY, J. P., JONES, P. D., AND FUYING, Z. C. (1992). Integrated assessment of contaminated sediments in the lower Fox River and Green Bay, Wisconsin. *Ecotoxicol. Environ. Saf.* **23**, 46–63.

ASHBY, J., AND TENNANT, R. W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229–306.

ASPLUND, G., GRIMVALL, A., AND PETTERSSON, C. (1989). Naturally produced adsorbable organic halogens (AOX) in humic substances from soil and water. *Sci. Total Environ.* **81/82**, 239–248.

BANERJEE, S., YALKOWSKY, S. H., AND VALVANI, S. C. (1980). Water solubility and octanol/water partition coefficients of organics. Limitations of the solubility–partition coefficient correlation. *Environ. Sci. Technol.* **14**(10), 1227–1229.

BARBIN, A., BRÉSIL, H., CROISY, A., JAQUIGNON, P., MALAVEILLE, C., MONTESANO, R., AND BARTSCH, H. (1975). Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* 67(2), 596–603.

BECK, H., AND HEINRICH-HIRSCH, B. (1993). *Organochlorverbindungen in Frauemilch. Bundesgesundheitsble 36.* Submitted.

BERGMAN, A., AND OLSSON, M. (1985). *Pathology of Baltic Grey Seal and Ringed Seal Females with Special Reference to Adrenocortical Hyperplasmia: Is Environmental Pollution the Cause of a Widely Distributed Disease Syndrome?* Proceedings from the Symposium on the Seals in the Baltic and Eurasian Lakes. June 5–8, 1984, Savonlinna, Finland. Part I: Seals in the Baltic.

BERLIN, W. H., HESSELBERG, R. J., AND MAC, M. J. (1981). Growth and mortality of fry of Lake Michigan Lake trout during chronic exposure to PCBs and DDE. In *Chlorinated Hydrocarbons as a Factor in the Reproduction and Survival of Lake Trout in Lake Michigan.* pp. 11–22. U.S. Fish and Wildlife Technical Paper 105, Fort Collins, CO.

BISHOP, C., AND WESELOH, D. V. (1990). *Contaminants in Herring Gulls Eggs from the Great Lakes.* Minister of Supply and Services Canada. Catalogue No. EN1-12/90-2E. ISBN 0-662-17922-6.

BONAVENTURA, J., AND BONAVENTURA, C. (1987). Carolina fish may be toxin carriers. *Environs.* **10**(3), 1; cited in Geraci, J. R. (1989).

BORTONE, S. A., AND DRYSDALE, D. T. (1981). Additional evidence for environmentally induced intersexuality in poeciliid fishes. *Assoc. Southeastern Biol. Bull.* **28**, 67.

BOSVELD, B. A. T. C., VAN DEN BERG, M., AND THEELEN, R. M. C. (1992). Assessment of the EROD inducing potency of eleven 2,3,7,8-substituted PCDD/Fs and three coplanar PCBs in the chick embryo. *Chemosphere* **25**, 911–916.

BRO-RASMUSSEN, F., AND LOKKE, H. (1984). Ecoepidemiology-A casuistic discipline describing ecological disturbances in relation to their specific causes: Exemplified by chlorinated phenols and Chlorophenoxy Acids. *Regul. Toxicol. Pharmacol.* **4**, 391–399.

BROUWER, A., REIJNDERS, P. J. H., AND KOEMAN, J. H. (1989). Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (*Phoca vitulina*). *Aquat. Toxicol.* **15**, 99–105.

BUMB, R. R., CRUMMETT, W. B., CUTIE, S. S., GIEDHILL, J. R., HUMMEL, R. H., KAGEL, R. O., LAMPARSKI, L. L., LUOMA, E. V., MILLER, D. L., NESTRICK, T. J., SHADOFF, L. A., STEHL, R. H., AND WOODS, J. S. (1980). Trace chemistries of fire: A source of chlorinated dioxins. *Science* **210**, 385–390.

CABRAL, J. R. P., HALL, R. K., ROSSI, L., BRONCYZK, S. A., AND SHUBIK, P. (1982). Effects of long-term intake of DDT on rats. *Tumorigenesis* **68**, 11–17.

CEPA (Canadian Environmental Protection Act) (1990). *Priority Substances List. Polychlorinated Debenzodioxins and Polychlorinated Debenzofurans.* Assessment Report No. 1. Canadian Environmental Protection Act. Environmental Canada, Ottawa, Ontario.

CASARETT, L. J., FRYER, G. C., YAUGER, W. L., AND KLEMMER, H. (1968). Organochlorine pesticide residues in human tissue—Hawaii. *Arch. Environ. Health* **17**, 306–311.

CHOU, I. T., AND JURS, P. C. (1979). Computer-assisted structure–activity studies on chemical carcinogens. An *N*-nitroso compound. *J. Med. Chem.* 22, 792–797.

CHOU, S. F. J., AND GRIFFIN, R. A. (1986). Solubility and soil mobility of polychlorinated biphenyls. In *PCBs and the Environment* (J. S. Waid, ed.), pp. 101–120. CRC Press, Boca Raton, Florida. 101–120.

CLEMENT, R. E., TOSINE, H. B., AND ALI, B. (1985). Levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in wood burning stoves and fireplaces. *Chemosphere* **14**, 815–819.

Clement Associates (1989). *Toxicological Profile for 1,2-Dichloroethane.* NTIS, PB90-171422. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

COURTNEY, K. D., COPELAND, M. F., AND ROBBINS, A. (1976). The effects of pentachloronitrobenzene, hexachlorobenzene and related compounds on fetal development. *Toxicol. Appl. Pharmacol.* **35**, 239–256.

CRAIG, P. N., AND ENSLEIN, K. (1981). Structure–activity in hazard assessment. In *Hazard Assessment of Chemicals.* Vol. 1. *Current Development.* Academic Press, San Diego.

CZUCZWA, J. M., MCVEETY, B. D., AND HITES, R. A. (1984). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Siskiwit Lake, Isle Royale. *Science* **226**, 568–569.

CZUCZWA, J. M., AND HITES, R. A. (1985). Dioxins and dibenzofurans in air, soil and water. In *Dioxins in the Environment* (M. A. Kamrin and P. W. Rodgers, eds.). Hemisphere Publishing Corp., Washington, DC.

DAR, E., KANAREK, M. S., ANDERSON, H. A., AND SONZOGNI, W. C. (1992). Fish consumption and reproductive outcomes in green bay Wisconsin. *Environ. Res.* **59**, 189–201.

DAVIS, W. P. (1989). *Review of the Androgenic Effects on Poeciliid and Other Fishes Living in Streams Receiving Kraft Mill Effluents.* EPA/600/X-89/18. (U.S.) Environmental Protection Agency. Gulf Breeze, Florida.

DEAN, J. H., AND MURRAY, M. J. (1991). Toxic Responses of the Immune System. In *Casarett and Doull's Toxicology: The Basic Science of Poisons* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.). Pergamon Press, Elmsford, NY.

DIETZ, R., HEIDE-JORGENSEN, M.-P., AND HARKONEN, T. (1989). Mass deaths of harbor seals (*Phoca vitulina*) in Europe. *Ambio* **18**(5), 258–264.

DOLL, R. (1988). Effects of exposure to vinyl chloride. *Scand. J. Work Environ. Health* **14**, 61–78.

EDER, E., NEUDECKER, T., LUTZ, D., AND HENSCHLER, D. (1980). *Biochem. Pharmacol.* **29**, 933; cited in Woo *et al.* (1985).

EITZER, B. D., AND HITES, R. A. (1989). Atmospheric transport and deposition of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Environ. Sci. Technol.* **23**, 1396–1401.

ENSLEIN, K., AND CRAIG, P. N. (1982). Carcinogenesis: A predictive structure-activity mode. *J. Toxicol. Environ. Health* **10**, 521–530.

Environment Canada (1991a). *Toxic Chemicals in the Great Lakes and Associated Effects: Synopsis.* Environment Canada, Government of Canada, Ottawa, Ontario.

Environment Canada (1991b). *Toxic Chemicals in the Great Lakes and Associated Effects.* Vol. I—*Contaminant Levels and Trends.* Environment Canada, Government of Canada, Ottawa, Ontario.

Environment Canada (1991c). *Toxic Chemicals in the Great Lakes and Associated Effects.* Vol. II—*Effects.* Environment Canada, Government of Canada, Ottawa, Ontario.

Environment Canada (1991d). *Effluents from Pulp Mills Using Bleaching.* Priority substances list. Assessment Report No. 2. Canadian Environmental Protection Act.

EPA (1985). *Health Assessment Document for Polychlorinated Dibenzo-p-dioxins.* NTIS PB86-122546. (U.S.) Environmental Protection Agency, Cincinnati, OH.

EPA (1988). *Drinking Water Criteria Document for Polychlorinated Biphenyls (PCBs).* (Final), PB89-192256. April 1988. Environmental Protection Agency, Cincinnati, OH.

EXON, J. H., KERKVLIET, N. I., AND TALCOTT, P. A. (1987). Immunotoxicity of carcinogenic pesticides and related chemicals. *Environ. Carcin. Rev. C* **5**(1), 73–120.

FALCK, F., RICCI, A., JR., WOLFF, M. S., GODBOLD, J., AND DECKERS, P. (1992). Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. Environ. Health* **47**(2), 143–146.

FEIN, G. G., JACOBSON, J. L., JACOBSON, S. W., SCHWARTZ, P. W., AND DOWLER, J. K. (1984). Prenatal exposure to polychlorinated biphenyls: Effects on birth size and gestational age. *J. Pediatr.* **105**, 315–320.

FENICAL, W. (1981). Natural Halogenated Organics. In *Marine Organic Chemistry. Evolution, Composition, Interactions and Chemistry of Organic Matter in Sea Water* (E. K. Duursma, and R. Dawson, eds.), pp. 375–393. Elsevier Scientific, Amsterdam.

FITZGERALD, E. F., HWANG, S., BRIX, K. A., BUSH, B., QUINN, J., AND COOK, K. (1992). *Chemical Contaminants in the Milk of Mohawk Women from Akwesasne.* N.Y. State Dept. of Health.

FOX, G. A. (1991). Practical causal inference for ecoepidemiologists. *J. Toxicol. Environ. Health* 33, 359–373.

FOX, G. A., COLLINS, B., HAYAKAWA, E., WESELOH, D. V., LUGWIG, J. P., KUBIAK, T. J., AND ERDMAN, T. C. (1991). Reproductive outcomes in colonial fish-eating birds: A biomarker for developmental toxicants in Great Lakes food chains. *J. Great Lakes Res.* 17(2), 158–167.

FRANKS, N. P., AND LIEB, W. R. (1982). Molecular mechanisms of general anaesthesia. *Nature* **300**, 487–493.

FRANKS, N. P., AND LIEB, W. R. (1984). Do general anaesthetics act by competitive binding to specific receptors? *Nature* **310**, 599–601.

FRIERSON, M. R., KLOPMAN, G., AND ROSENKRANZ, H. S. (1986). Structure–activity relationships (SARs) among mutagens and carcinogens: A review. *Environ. Mutagen.* **8**, 283–327.

GERACI, J. R., ST. AUBIN, D. J., BARKER, I. K., WEBSTER, R. G., HINSHAW, V. S., BEAN, W. J., RUHNKE, H. L., PRESCOTT, J. H., EARLY, G., BAKER, A. S., MADOFF, S., AND SCHOOLEY, R. T. (1982). Mass mortality of harbor seals: Pneumonia associated with influenza A virus. *Science* **215**(26), 1129.

GERACI, J. R., ST. AUBIN, D. J., BARKER, I. K., HINSHAW, V. S., WEBSTER, R. G., AND RUHNKE, H. L. (1984). Susceptibility of grey (*Halichoerus grypus*) and harp (*Phoca groenlandica*) seals to the influenza virus and mycoplasm of epizootic pneumonia of harbor seals (*Phoca vitulina*). *Can. J. Fish Aquat. Sci.* **41**, 151–156.

GERACI, J. R. (1989). *Clinical Investigation of the 1987–1988 Mass Mortality of Bottlenose Dolphins Along the U.S. Central and South Atlantic Coast.* Final Report to National Marine Fisheries Service and (U.S.) Navy. Wildlife Disease Section, Ontario Veterinary College, Guelph, Ontario.

GILBERTSON, M. (1983). Etiology of chick-edema disease in herring gulls in the lower Great Lakes. *Chemosphere* **12**, 357–370; cited in Gilbertson *et al.* (1991).

GILBERTSON, M., KUBIAK, T., LUDWIG, J., AND FOX, G. (1991). Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: Similarity to chick-edema disease. *J. Toxicol. Environ. Health* **33**, 455–520.

GRACHEV, M. A., KUMAREV, V. P., MAMAEV, L. V., ZORIN, V. L., BARANOVA, L. V., DENIKINA, N. N., BELIKOV, S. I., PETROV, E. A., KOLESNIK, V. S., KOLESNIK, R. S., DOROFEEV, V. M., BEIM, A. M., KUDELIN, V. N., NAGIEVA, F. G., AND SDIOROV, V. N. (1989). Distemper virus in Baikal seals. *Nature* **338**, 209.

GRIBBLE, G. (1992). Naturally occurring organohalogen compounds: A survey. *J. Nat. Prod.* **55**(10), 1353–1395.

GRIER, J. W. (1982). Ban of DDT and subsequent recovery of reproduction in bald eagles. *Science* **218**, 1232–1235.

HANSCH, C., AND LEO, A. (1979). *Substituent Constants for Correlation Analysis in Chemistry and Biology.* Wiley, New York.

HAQUE, R., AND SCHMEDDING, D. W. (1976). Studies of adsorption of selected polychlorinated biphenyls isomers on several surfaces. *J. Environ. Sci. Health B* **11**, 129; cited in Chou and Griffin (1986).

HAQUE, R., SCHMEDDING, D. W., AND FREED, V. H. (1974). Aqueous solubility, adsorption, and vapor behavior of polychlorinated biphenyl Aroclor 1254. *Environ. Sci. Technol.* **8**, 139; cited in Sawhney (1986).

HARPER, D. B. (1985). Halomethane from halide ion—A highly efficient fungal conversion of environmental significance. *Nature* **315**(6014), 55–57.

HARWOOD, J., CARTER, S. D., HUGHES, D. E., BELL, S. C., BAKER, J. R., AND CORNWELL, H. J. C. (1989). Seal disease predictions. *Nature* **339**, 670.

HAYES, W. J., JR. (1982). *Pesticides Studied in Man.* Williams and Wilkins, Baltimore.

HAYS, H., AND RISEBROUGH, R. W. (1972). Pollutant concentrations in abnormal young terns from Long Island Sound. *Auk* **89**, 19–35; cited in Gilbertson *et al.* (1991).

HENSCHLER, D. (1985). Halogenated Alkenes and Alkynes. In *Bioactivation of Foreign Compounds* (M. W. Anders, ed.), pp. 317–347. Academic Press, New York.

HENSCHLER, D. (1990). Science, occupational exposure limits, and regulations: A case study on organochlorine solvents. *Am. Ind. Hyg. Assoc. J.* **51**(10), 523–530.

HEWITT, L. M., CAREY, J. H., MUNKITTRICK, K. R., AND DIXON, D. G. (1992). *Isolation of EROD Inducing Material from Treated Kraft Mill Effluents.* Presented at the 19th Annual Aquatic Toxicity Workshop, Edmonton, Alberta, October 4–7, 1992.

HILL, A. B. (1965). The environment and disease: Association or Causation? *R. Soc. Med.* 295–300.

HINSHAW, V. S., BEAN, W. J., WEBSTER, R. G., REHG, J. E., FIORELLI, P., EARLY, G., GERACI, J. R., AND ST. AUBIN, D. J. (1984). Are seals frequently infected with avian influenza viruses? *J. Virol.* 51(3), 863–865.

HITES, R. W. (1990). Environmental behavior of chlorinated dioxins and furans. *Acc. Chem. Res.* 23, 194–201.

HODSON, P. V., MCWHIRTER, M., RALPH, K., GRAY, B., THIVERGE, D., CAREY, J. H., VAN DER KRAAK, G., WHITTLE, D. M., AND LEVESQUE, M.-C. (1992a). Effects of bleached kraft mill effluent on fish in the St. Maurice River, Quebec. *Environ. Toxicol. Chem.* 11, 1635–1651.

HODSON, P. V., PARROTT, J., AND MACLEAN, R. (1992b). *Fish Liver Mixed Function Oxygenases (MFOs) as Indicators of Effluent Exposure and Fish Health.* Presented as the 7th Colloquium on Pulp and Paper Mill Effluents. December 15 & 16, 1992. Pulp and Paper Centre, University of Toronto, Toronto, Ontario, Canada.

HOFFMAN, W. S., ADLER, H., FISHBEIN, W. I., AND BAUER, F. C. (1967). Relation of pesticide concentration in fate to pathological changes in tissues. *Arch. Environ. Health* 15, 758–765.

HOFFMAN, D. J., RATTNER, B. A., SILEO, L., DOCHERTY, D., AND KUBIAK, T. J. (1987). Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environ. Res.* 42, 176–184.

HOWELL, W. M., BLACK, A., AND BORTONE, S. A. (1980). Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki;* Evidence for environmentally induced masculinization. *Copeia,* 676–681.

HUHEEY, J. E. (1965). The electronegativity of groups. *J. Phys. Chem.* 69, 3284–3291.

HWC (Health and Welfare Canada) (1988). *Chlorophenols and Their Impurities: A Health Hazard Evaluation.* Health and Welfare Canada, Ottawa, Ontario.

IDSP (Industrial Disease Standards Panel) (1987). Report to the Workers' Compensation Board on Occupational Exposure to PCBs. IDSP Report No. 2. Toronto, Ontario.

ITO, N., TSUDA, H., HASEGAWA, R., AND IMAIDA, K. (1982). Sequential observation of pathomorphological alterations in preneoplastic lesions during the promoting stage of hepatocarcinogenesis and the development of short-term test system for hepatopromoters and hepatocarcinogens. *J. Toxicol. Pathol.* 10(2), 37–49; cited in EPA (1986).

JACOBSON, J. L., FEIN, G. G., JACOBSON, S. W., SCHWARTZ, P. M., AND DOWLER, J. K. (1984). The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls across the human placenta and into maternal milk. *Am. J. Public Health* 74, 378–379.

JACOBSON, J. L., HUMPHREY, H. E. B., JACOBSON, S. W., SCHANTZ, S. L., MULLIN, M. D., AND WELCH, R. (1989). Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. *Am. J. Public Health* 79(10), 1401–1404.

JACOBSON, J. L., JACOBSON, S. W., AND HUMPHREY, H. E. B. (1990a). Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. *J. Pediatr.* 116, 38–45.

JACOBSON, J. L., JACOBSON, S. W., AND HUMPHREY, H. E. B. (1990b). Effects of exposure to PCBs and related compounds on growth and activity in children. *Neurotoxicol. Teratol.* 12, 319–326.

JEFFERS, P. M., WARD, L. M., WOYTOWITCH, L. M., AND WOLFE, N. L. (1989). Homogeneous hydrolysis rate constants for selected chlorinated methanes, ethanes, ethenes, and propanes. *Environ. Sci. Technol.* 23(8), 965–969.

JOHANNSEN, F. J. (1990). Risk assessment of carcinogenic and noncarcinogenic chemicals. *Crit. Rev. Toxicol.* 20(5), 341–367.

JURS, P. C., CHOW, J. T., AND YUAN, M. (1979). Computer-assisted structure–activity studies of chemical carcinogens. A heterogeneous data set. *J. Med. Chem.* 22, 476–483.

KIMBROUGH, R. D., SQUIRE, R. A., LINDER, R. E., STRANDBERG, J. D., MONTALI, R. J., AND BURSE, V. W. (1975). Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. *J. Natl. Cancer Inst.* 55, 1453–1459.

KLAASSEN, C. D., AND EATON, D. L. (1991). Principles of Toxicology. In *Casarett and Doull's Toxicology: The Basic Science of Poisons* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.) Pergamon Press, Elmsford, NY.

KLOPMAN, G. (1984). Artificial intelligence approach to structure–activity studies. Computer automated structure evaluation of biological activity or organic molecules. *J. Am. Chem. Soc.* 106, 7315–7321.

KLOPMAN, G., BALTHASAR, D. M., AND ROSENKRANZ, H. S. (1993). Application of the computer-automated structure evaluation (CASE) program to the study of structure–biodegradation relationships of miscellaneous chemicals. *Environ. Toxicol. Chem.* **12**, 231–240.

KLOPMAN, G., NAMBOODIRI, K., AND KALOS, A. N. (1985). Computer Automated Evaluation and Prediction of the Iball Index of Carcinogenicity of PAHs. In *Molecular Basis of Cancer. Part A: Marcomolecular Structure, Carcinogens, and Oncogenes* (R. Rein, ed.). A. R. Liss, New York.

KOCIBA, R. J., KEYES, D. G., BEYER, J. E., CARRESON, R. M., WADE, E. E., DITTENBER, D. A., KALMINS, R. P., FRAUSON, L. F., PARK, D. N., BERNARD, S. D., HUMMEL, R. A., AND HUMISTON, C. G. (1978). Results of a two year chronic toxicity and oncogenicity study or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (tetraCDD) in rats. *Toxicol. Appl. Pharmacol.* **46**, 279–303.

KREISS, K., ZACK, M. M., KIMBROUGH, R. D., NEEDHAM, L. L., SMREK, A. L., AND JONES, B. T. (1981). Association of blood pressure and polychlorinated biphenyl levels. *J. Am. Med. Assoc.* **245**, 2505–2509.

KUBIAK, T. J., HARRIS, H. J., SMITH, L. M., SCHWARTZ, T. R., STALLING, D. L., TRICK, J. A., SILEO, L., DOCHERTY, D. E., AND ERDMAN, T. C. (1989). Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan—1983. *Arch. Environ. Contam. Toxicol.* **18**, 706–727.

KURITA, H., LUDWIG, J. P., AND LUDWIG, M. E. (1987). *Results of the 1987 Michigdn Colonial Waterbird Monitoring Project on Caspian Terns and Double-Crested Cormorants: Egg Incubation and Field Studies of Colonial Productivity, Embryonic Mortality and Deformities.* Special Report to the Michigan Department of Natural Resources, Ecological Research Services Inc., September 30, 1987.

LARSSON, L., ANDERSSON, T., FORLIN, L., AND HARDIG, J. (1988). Physiological disturbances in fish exposed to bleached kraft mill effluents. *Water Sci. Technol.* 20(2), 67–76.

LINDSTROM-SEPPA, P., AND OIKARI, A. (1989). Bleached kraft pulpmill effluents and TCDD: Effects on biotransformation and testosterone status of mature male rainbow trout (*Salmo gairdneri*). *Environ. Res.* 28, 99–103.

LINDSTROM-SEPPA, P., AND OIKARI, A. (1990a). Biotransformation and other toxicological and physiological responses in rainbow trout (*Salmo gairdneri* Richardson) caged in a lake receiving effluents of pulp and paper industry. *Aquat. Toxicol.* **16**(1), 187–204.

LINDSTROM-SEPPA, P., AND OIKARI, A. (1990b). Biotransformation activities of feral fish in waters receiving bleached pulp mill effluents. *Environ. Toxicol. Chem.* 9, 1415–1424.

LOCKHART, W. L., AND METNER, D. A. (1992). *Cytochrome P-450-Related Enzymatic Activities in Fish from Western Canadian Rivers Receiving Chlorinated Kraft Pulp Mill Wastes.* Presented at the 19th Annual Aquatic Toxicity Workshop, October 4–7, 1992, Edmonton, Alberta, Canada.

MABEY, W., AND MILL, T. (1978). Critical review of hydrolysis of organic compounds in water under environmental conditions. *J. Phys. Chem. Ref. Data* 7(2), 383–415.

MAC, M. J., EDSAL, C. C., AND SEELYE, J. G. (1985). Survival of lake trout eggs and fry reared in water from the upper Great Lakes. *J. Great Lakes Res.* **11**, 520–529.

MACKAY, D. (1992). Is chlorine really the evil element? *Environ. Sci. Engr.* 49–55.

MACKAY, D., SHIU, W. Y., AND MA, K. C. (1992). *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals. Vol. I. Monoaromatic Hydrocarbons, Chlorobenzenes, and PCBs.* Lewis, Chelsea, MI.

MANSON, J. M., AND WISE, L. D. (1991). Teratogens. In *Casarett and Doull's Toxicology: The Basic Science of Poisons,* 4th ed., (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.), pp. 127–225. Pergamon Press, New York.

MARK, A. J., VAN DER BERG, J. H. J., KOEMAN, J. H., AND BROUWER, A. (1990). Comparative Toxicity Study of Tetrachlorobenzltoluenes (UGILEC 141) and Polychlorobiphenyls (AROCLOR 1254 and PCB-77) in AB-Responsive (C578L/6) and AB-Nonresponsive (DBA/2) mice. In *Dioxin'90 EPRI—Seminar. Short Papers. Toxicology, Environment Food, Exposure—Risk* (O. Hutzinger and H. Fielder, eds.), pp. 199–203. Ecoinforma Press, Bayreuth, FRG.

MARKS, T. A., KIMMEL, G. L., AND STAPLES, R. E. (1981). Influence of symmetrical polychlorinated biphenyl isomers on embryo and fetal development in mice. *Toxicol. Appl. Pharmacol.* **61**, 269–276.

MARTEL, P., AND KOVACS, T. (1992). *Relationship between Mill Processes and the Potential of Pulp and Paper Mill Effluents to Induce Mixed Function Oxygenase Activity in Fish.* Presented as the 7th Colloquium on Pulp and Paper Mill Effluents, December 15 and 16, 1992, Pulp and Paper Centre, University of Toronto, Toronto, Ontario, Canada.

MASLANSKY, C. J., AND WILLIAMS, G. M. (1981). Evidence for an epigenic mode of action in organochlorine pesticide hepatocarcinogenicity: A lack of genotoxicity in rat, mouse, and hamster hepatocytes. *J. Toxicol. Environ. Health* 19, 577–583.

MATTHEWS, H. B., AND DEDRICK, R. L. (1984). Pharmacokinetics of PCBs. *Annu. Rev. Pharmacol. Toxi* 24, 85–103.

MAYER, K. S., MAYER, F. L., AND WITT, A. (1985). Waste transformer oil and PCB toxicity to raint trout. *Trans. Am. Fish Soc.* **114,** 869–886.

MCEWEN, F. L., AND STEPHENSON, G. R. (1979). *The Use and Significance of Pesticides in the Environm* Wiley, New York.

MCLEAY, D., AND Associates Ltd. (1987). *Aquatic Toxicity of Pulp and Paper Mill Effluent: A Rev:* Environment Canada, Ottawa. Report EPS 4/PF/1.

MCMASTER, M. E., VAN DER KRAAK, G. J., PORTT, C. B., MUNKITTRICK, K. R., SIBLEY, P. K., SMI I. R., AND DIXON, D. G. (1991). Changes in hepatic mixed function oxygenase (MFO) activity, pla: steroid levels and age to maturity of a white sucker (*Catastomus commersoni*) population exposec bleached kraft pulp mill effluent. *Aquat. Toxicol.* **21,** 199–218.

MINEAU, P., FOX, G. A., NORSTROM, R. J., WESELOH, D. V., HALETT, D. J., AND ELLENTON, J. A. (19 Using the Herring Gull to Monitor Levels and Effects of Organochlorine Contaminants in the Canac Great Lakes. In *Toxic Contaminants in the Great Lakes. Advances in Environmental Science and Toxico.* (J. O. Nriagu and M. S. Simmons, eds.), Vol. 14, pp. 425–452.

MOE (Ministry of the Environment) (1985). *Polychlorinated Dibenzo-p-dioxins (PCDDs) and Polychlorin. Dibenzofurans (PCDFs).* Ministry of the Environment, Ontario, Scientific Criteria Document for Stanc Development No. 4-84.

MOORE, D. R. J., WALKER, S. L., AND KONIECKI, D. (1991). *Canadian Water Quality Guidelines Chlorinated Ethanes.* Scientific Series No. 185. Inland Waters Directorate, Water Quality Branch, E ronment Canada, Ottawa. ISBN 0-662-19185-4.

MUIR, D. C. G., YARECHEWSKI, A. L., METNER, D. A., LOCKHART, W. L., WEBSTER, G. R. B., FRIESEN, K. J. (1990). Dietary accumulation and sustained hepatic mixed function oxygenase enz induction by 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. *Environ. Toxicol. Chem.* 9, 14 1472.

MUNKITTRICK, K. R., PORTT, C. B., VAN DER KRAAK, G. J., SMITH, I. R., AND ROKOSH, D. A. (19 *Impact of Bleached Kraft Mill Effluent on Liver MFO Activity, Serum Steroids and White Sucker Popula Characteristics.* Abstract No. 333, Abstracts of the 10th Annual Meeting of the Society of Environme Toxicology and Chemistry. Toronto, Ontario. (October 28–November 2, 1989); cited in Environn Canada (1991).

MUNKITTRICK, K. R., PORTT, C. B., VAN DER KRAAK, G. J., SMITH, I. R., AND ROKOSH, D. A. (19 Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum ste levels of a Lake Superior White Sucker (*Catostomus commersoni*) population. *Can. J. Fish Aquat.* **48,** 1371–1380.

MUNKITTRICK, K. R., VAN DER KRAAK, G. J., MCMASTER, M. E., AND PORTT, C. B. (1992a). Resp of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft pulp mill effl and mill shutdown. *Environ. Toxicol. Chem.* **11,** 1427–1439.

MUNKITTRICK, K. R., LUXON, P. L., MCMASTER, M. E., JARDINE, J. J., AND VAN DER KRAAK, ((1992b). *Impact of Exposure to Kraft Mill Effluent and Effect of Low Level Sampling Stress on Interpreta of Non-specific Indicators of Exposure.* Presented as the 7th Colloquium on Pulp and Paper Mill Efflu December 15 and 16, 1992, Pulp and Paper Centre, University of Toronto, Ontario, Canada.

MUNRO, I. C., CARLO, G. L., SUND, K. G., WILSON, R. M., KENNEPOHL, E., LYNCH, B. S., JABLIN M., AND LEE, N. L. (1992). A comprehensive, integrated review and evaluation of the scientific evid relating to the safety of the herbicide 2,4-D. *J. Am. Coll. Toxicol.* **11**(5), 559–664.

NCASI (National Council of the Paper Industry for Air and Stream Improvement) (1992). *Naturally Oc ring Organohalogen Compounds—A Survey.* National Council of the Paper Industry for Air Stream Improvement, 290 Madison Ave., New York, NY 10016. Technical Bulletin No. 629, M: 1992.

NESTRICK, T. J., AND LAMPARSKI, L. L. (1982). Isomer-specific determination of chlorinated dioxin: assessment of formation and potential environmental emission from wood combustion. *Anal. Chem* 2292–2299; cited in Sheffield (1985).

NEWTON, I., BOGAN, J. A., AND HAAS, M. B. (1989). Organochlorines and mercury in the eggs of Br peregrines *Falco peregrinus. Ibis* **131,** 355–376.

NOBLE, D. G., AND ELLIOT, J. E. (1986). *Environmental Contaminants in Canadian Seabirds 1968–1 Trends and Effects.* Technical Report Series No. 13, p. 275. Canadian Wildlife Service, Ottawa.

OBERG, L. G., GLAS, B., AND RAPPE, C. (1991). *Biochemical Formation of PCDDs and PCDFs.* Eleventh International Symposium on Chlorinated Dioxins and Related Compounds. September 23–27, 1991, Research Triangle Park, North Carolina, pp. 19.

OLSEN, S. F., OLSEN, J., AND FRISCHE, G. (1990). Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. *Int. J. Epidemiol.* **19**(4), 971–977.

OSTERHAUS, A. D. M. E., UYTDEHAAG, F. G. C. M., VISSER, I. K. G., VEDDER, E. J., REIJNDERS, P. J. H., KUIPER, J., AND BRUGGE, H. N. (1989). Seal vaccination success. *Nature* **337**, 21.

OSTP (Office of Science and Technology Policy) (1985). Chemical carcinogens: A review of the science and associated principles. *Fed. Regist.* **50**, 10372.

PANETH, N. (1991). Human reproduction after eating PCB-contaminated fish. *Health Environ. Dig.* **5**(8), 4–6.

PAVLOU, S. P., AND DEXTER, R. N. (1979). Distribution of polychlorinated biphenyls (PCB) in estuarine ecosystems. Testing the concept of equilibrium partitioning in the marine environment. *Environ. Sci. Technol.* **13**, 65; cited in Phillips (1986).

PAYNE, J. F., FANCEY, L. L., RAHIMTULA, A. D., AND PORTER, E. L. (1987). Review and perspective on the use of mixed-function oxygenase exzymes in biological monitoring. *Comp. Biochem. Physiol. C* **86**, 233–245.

PHILLIPS, D. J. H. (1986). Use of Organisms to Quantify PCBs in Marine and Estuarine Environments. In *PCBs and the Environment* (J. S. Waid, ed.), pp. 127–182. CRC Press, Boca Raton, Florida.

PIGNATELLO, J. J., MARTINSON, M. M., STEIERT, J. G., CARLSON, R. E., AND CRAWFORD, R. L. (1983). Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. *Appl. Environ. Microbiol.* **46**(5), 1024–1031.

POLAND, A. (1984). Reflections on the Mechanism of Action of Halogenated Aromatic Hydrocarbons. In *Banbury Report 18: Biological Mechanisms of Dioxin Action* (A. Poland and R. D. Kimbrough, eds.), pp. 109–118. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

POLITZER, P., AND LAURENCE, P. R. (1984). Relationship between the electrostatic potential, epoxide hydrase inhibition and carcinogenicity for some hydrocarbon and halogenated hydrocarbon epoxides. *Carcinogenesis* **5**(6), 845–848.

PRICE, I. M., AND WESELOH, D. V. (1986). Increased number and productivity of double-crested cormorant, *Phalacrocorax auritus* on Lake Ontario. *Can. Field Natur.* **100**(4), 474–482.

RADOMSKI, J. L., DEICHMANN, W. B., AND CLIZER, E. E. (1968). Pesticide concentrations in the liver brain and adipose tissue of terminal hospital patients. *Food Cosmet. Toxicol.* **6**, 209–220.

RAND, G. M., AND PETROCELLI, S. R. (eds.) (1984). *Fundamentals of Aquatic Toxicology.* Hemisphere Publishing Corp., New York.

RAY, D. E. (1991). Pesticides Derived for Plants and Other Organisms. In *Handbook of Pesticide Toxicology* (Hayes and Laws, ed.), Vol. 2: Classes of Pesticides. Academic Press, San Diego.

Regulatory Network, Inc. (1992). *Documenting Temporal Trends of Polychlorinated Biphenyls in the Environment (Draft).* Prepared for Chemical Manufacturers Association, PCB Panel, National Electric Manufacturer's Association.

REHANA, T., AND RAO, P. R. (1992). Effect of DDT on the immune system in swiss albino mice during adult and perinatal exposure: Humoral responses. *Bull. Environ. Contam. Toxicol.* **48**, 535–540.

REIJNDERS, P. J. H. (1986). Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **324**, 456–457.

ROBINSON, P. E., MACK, G. A., REMMERS, J., LEVY, R., AND MOHADJER, L. (1990). Trends of PCB, hexachlorobenzene, and beta-benzene hexachloride levels in the adipose tissue of the U.S. population. *Environ. Res.* **53**(2), 175–192.

ROGAN, W. J., GLADEN, B. C., MCKINNEY, J. D., CARRERAS, N., HARDY, P., THULLEN, J., TINGELSTAD, J., AND TULLY, M. (1986a). Polychlorinated biphenyls (PCBs) and dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. *Am. J. Public Health* **76**, 172–176.

ROGAN, W. J., GLADEN, B. C., MCKINNEY, J. D., CARRERAS, N., HARDY, P., THULLEN, J., TINGLESTAD, J., AND TULLY, M. (1986b). Neonatal effects of transplacental exposure to PCBs and DDE. *J. Pediatr.* **109**, 335–341.

ROGERS, I. H., LEVINGS, C. D., LOCKHART, W. L., AND NORSTROM, R. J. (1989). Observations on overwintering juvenile chinook salmon (*Oncorhynchus tshawytscha*) exposed to bleached kraft mill effluent in the upper Fraser River, British Columbia. *Chemosphere* **19**(12), 1853–1868.

ROSE, S. L., AND JURS, P. C. (1982). Computer-assisted studies of structure-activity relationships of / nitroso compounds using pattern recognition. *J. Med. Chem.* **25**, 769–776.

ROSSI, L., RAVERA, M., REPETTI, G., AND SANTI, L. (1977). Long-term administration of DDT or ph nobarbital-Na in Wistar rats. *Int. J. Cancer* **19**, 179–185.

SAFE, S. (1989). Polychlorinated biphenyls (PCBs): Mutagenicity and carcinogenicity. *Mutat. Res.* **220**, 3 47.

SALKINOJA-SALONEN, M., SAXELIN, M.-L., PERE, J., JAAKKOLA, T., SAARIKOSKI, J., HAKULINEN, R., AI KOISTINEN, O. (1984). Analysis of toxicity and biodegradability of organochlorine compounds releas into the environment in bleaching effluents of Kraft Pulping. In *Advances in the Identification and Analy of Organic Pollutants in Water* (L. H. Keith, ed.), Vol. 2, pp. 1131–1164. Ann Arbor, MI.

SCHELLENBERG, K., LEUENBERGER, C., AND SCHWARZENBACH, R. P. (1984). Sorption of chlorinated phen by natural sediment and aquifer materials. *Environ. Sci. Technol.* **18**(9), 652–657.

SCHOENHARD, G. L., LEE, D. J., HOWELL, S. E., PAWLOWSKI, N. E., LIBBEY, L. M., AND SIMMHUBE R. O. (1976). Aflatoxin B_1 metabolism to aflatoxicol and derivatives lethal to *Bacillus subtilis* GSY 10 by rainbow trout liver. *Cancer Res.* **36**, 2040–2045; cited in Rand and Petrocelli (1984).

SCHUMACHER, U., HORNY, H. P., HEIDEMANN, G., SKIRNISSON, K., AND WELSCH, U. (1991). Histologi and histochemical investigations into the cause of the recent seal death epidemic. *Prog. Histo.-Cytoche.* **23**, 390–394.

SERVOS, M., CAREY, J., FERGUSON, M., VAN DER KRAAK, G., FERGUSON, H., PARROTT, J., GORMAN, I AND COWLING, R. (1992). Impact of a modernized bleached kraft mill on white sucker populations the Spanish River, Ontario. *Water Pollut. Res. J. Can.*, in press.

SILBERHORN, E. M., GLAUERT, H. P., AND ROBERTSON, L. W. (1990). Carcinogenicity of polyhalogenat biphenyls: PCBs and PBBs. *Crit. Rev. Toxicol.* **20**(6), 440–496.

SILKWORTH, J. B., AND VECCHI, A. (1985). Role of the Ah receptor in halogenated aromatic hydrocarb immunotoxicity. In *Immunotoxicity and Immunopharmacology* (Dean, Luster, Munson, and Amos, ed: Raven Press, New York.

SIPES, I. G., AND GANDOLFI, A. J. (1991). Biotransformation of Toxicants. In *Casarett And Doull's Toxicolo The Basic Science of Poisons* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.), 4th ed., pp. 88–1 Pergamon Press, New York.

SIUDA, J. F., AND DEBERNARDIS, J. F. (1973). Naturally occurring halogenated organic compounds. *J. N Prod.* **36**(1–4), 107–143.

SMITH, I. R., AND ROKOSH, D. A. (1989). *An Epidemiological Study of a White Sucker (Catoston commersoni) Population Inhabiting the Kaministiquia River, Thunder Bay, Ontario.* Abstract No. 2 Abstracts of the 10th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Toron Ontario.

SÖDERGREN, A. (1989). *Biological Effects of Bleached Pulp Mill Effluents.* National Swedish Environmen Protection Board, Report 3558.

SÖDERGREN, A., BENGTSSON, B.-E. JONSSON, P., LAGERGREN, S., LARSON, Å., OLSSON, M., AND RENBEF L. (1988). Summary of results from the Swedish Project Environment/Cellulose. *Water Sci. Techn* **20**(1), 49–60.

SRC (Syracuse Research Corp.) (1989). *Toxicological Profile for Chloroform.* NTIS PB89-160360. U Agency for Toxic Substances and Disease Registry, Atlanta, GA.

STALLING, D. L., NORSTROM, R. J., SMITH, L. M., AND SIMON, M. (1985). Patterns of PCDD, PCDF a PCB contamination in Great Lakes fish and birds and their characterization by principal compon analysis. *Chemosphere* **14**, 627–643.

STEVENS, J. T., AND SUMNER, D. D. (1991). Herbicides. In *Handbook of Pesticide Toxicology. Vol. Classes of Pesticides* (Hayes and Laws, eds.). Academic Press, San Diego, CA.

STRUGER, J., AND WESELOH, D. V. (1985). Great Lakes Caspian terns: Egg contaminants and biologi implications. *Colonial Waterbirds* **8**, 142–149.

SUGIURA, K., AOKI, M., KANEKO, S., DAISAKU, I., KOMATSU, Y., SHIBUYA, H., SUZUKI, H., AND GO M. (1984). Fate of 2,4,6-trichlorophenol, pentachlorophenol, *p*-chlorobiphenyl, and hexachlorobenz in an outdoor experimental pond: Comparison between observations and predictions based on laborat data. *Arch. Environ. Contam. Toxicol.* **13**, 745–748.

TABAK, H. H., QUAVE, S. A., MASHNI, C. E., AND BARTH, E. F. (1981). Biodegradability studies with orga priority pollutant compounds. *J. Water Pollut. Control Fed.* **53**(10), 1503–1518.

TASHIRO, C., CLEMENT, R. E., STOCKS, B. J., RADKE, L., COFER, W. R., AND WARD, P. (1990). Prelimin report: Dioxins and furans in prescribed burns. *Chemosphere* **20**, 1533–1536.

ROSE, S. L., AND JURS, P. C. (1982). Computer-assisted studies of structure–activity relationships of *N*-nitroso compounds using pattern recognition. *J. Med. Chem.* **25**, 769–776.

ROSSI, L., RAVERA, M., REPETTI, G., AND SANTI, L. (1977). Long-term administration of DDT or phenobarbital-Na in Wistar rats. *Int. J. Cancer* **19**, 179–185.

SAFE, S. (1989). Polychlorinated biphenyls (PCBs): Mutagenicity and carcinogenicity. *Mutat. Res.* **220**, 31–47.

SALKINOJA-SALONEN, M., SAXELIN, M.-L., PERE, J., JAAKKOLA, T., SAARIKOSKI, J., HAKULINEN, R., AND KOISTINEN, O. (1984). Analysis of toxicity and biodegradability of organochlorine compounds released into the environment in bleaching effluents of Kraft Pulping. In *Advances in the Identification and Analysis of Organic Pollutants in Water* (L. H. Keith, ed.), Vol. 2, pp. 1131–1164. Ann Arbor, MI.

SCHELLENBERG, K., LEUENBERGER, C., AND SCHWARZENBACH, R. P. (1984). Sorption of chlorinated phenols by natural sediment and aquifer materials. *Environ. Sci. Technol.* **18**(9), 652–657.

SCHOENHARD, G. L., LEE, D. J., HOWELL, S. E., PAWLOWSKI, N. E., LIBBEY, L. M., AND SINNHUBER, R. O. (1976). Aflatoxin B_1 metabolism to aflatoxicol and derivatives lethal to *Bacillus subtilis* GSY 1057 by rainbow trout liver. *Cancer Res.* **36**, 2040–2045; cited in Rand and Petrocelli (1984).

SCHUMACHER, U., HORNY, H. P., HEIDEMANN, G., SKIRNISSON, K., AND WELSCH, U. (1991). Histological and histochemical investigations into the cause of the recent seal death epidemic. *Prog. Histo.-Cytochem.* **23**, 390–394.

SERVOS, M., CAREY, J., FERGUSON, M., VAN DER KRAAK, G., FERGUSON, H., PARROTT, J., GORMAN, K., AND COWLING, R. (1992). Impact of a modernized bleached kraft mill on white sucker populations in the Spanish River, Ontario. *Water Pollut. Res. J. Can.*, in press.

SILBERHORN, E. M., GLAUERT, H. P., AND ROBERTSON, L. W. (1990). Carcinogenicity of polyhalogenated biphenyls: PCBs and PBBs. *Crit. Rev. Toxicol.* **20**(6), 440–496.

SILKWORTH, J. B., AND VECCHI, A. (1985). Role of the Ah receptor in halogenated aromatic hydrocarbon immunotoxicity. In *Immunotoxicity and Immunopharmacology* (Dean, Luster, Munson, and Amos, eds.). Raven Press, New York.

SIPES, I. G., AND GANDOLFI, A. J. (1991). Biotransformation of Toxicants. In *Casarett And Doull's Toxicology: The Basic Science of Poisons* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.), 4th ed., pp. 88–126. Pergamon Press, New York.

SIUDA, J. F., AND DEBERNARDIS, J. F. (1973). Naturally occurring halogenated organic compounds. *J. Nat. Prod.* **36**(1–4), 107–143.

SMITH, I. R., AND ROKOSH, D. A. (1989). *An Epidemiological Study of a White Sucker (Catostomus commersoni) Population Inhabiting the Kaministiquia River, Thunder Bay, Ontario.* Abstract No. 262. Abstracts of the 10th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Toronto, Ontario.

SÖDERGREN, A. (1989). *Biological Effects of Bleached Pulp Mill Effluents.* National Swedish Environmental Protection Board, Report 3558.

SÖDERGREN, A., BENGTSSON, B.-E. JONSSON, P., LAGERGREN, S., LARSON, Å., OLSSON, M., AND RENBERG, L. (1988). Summary of results from the Swedish Project Environment/Cellulose. *Water Sci. Technol.* **20**(1), 49–60.

SRC (Syracuse Research Corp.) (1989). *Toxicological Profile for Chloroform.* NTIS PB89-160360. U.S. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

STALLING, D. L., NORSTROM, R. J., SMITH, L. M., AND SIMON, M. (1985). Patterns of PCDD, PCDF and PCB contamination in Great Lakes fish and birds and their characterization by principal component analysis. *Chemosphere* **14**, 627–643.

STEVENS, J. T., AND SUMNER, D. D. (1991). Herbicides. In *Handbook of Pesticide Toxicology. Vol. 3. Classes of Pesticides* (Hayes and Laws, eds.). Academic Press, San Diego, CA.

STRUGER, J., AND WESELOH, D. V. (1985). Great Lakes Caspian terns: Egg contaminants and biological implications. *Colonial Waterbirds* **8**, 142–149.

SUGIURA, K., AOKI, M., KANEKO, S., DAISAKU, I., KOMATSU, Y., SHIBUYA, H., SUZUKI, H., AND GOTO, M. (1984). Fate of 2,4,6-trichlorophenol, pentachlorophenol, *p*-chlorobiphenyl, and hexachlorobenzene in an outdoor experimental pond: Comparison between observations and predictions based on laboratory data. *Arch. Environ. Contam. Toxicol.* **13**, 745–748.

TABAK, H. H., QUAVE, S. A., MASHNI, C. E., AND BARTH, E. F. (1981). Biodegradability studies with organic priority pollutant compounds. *J. Water Pollut. Control Fed.* 53(10), 1503–1518.

TASHIRO, C., CLEMENT, R. E., STOCKS, B. J., RADKE, L., COFER, W. R., AND WARD, P. (1990). Preliminary report: Dioxins and furans in prescribed burns. *Chemosphere* **20**, 1533–1536.

TERNAY, A. L. (1979). *Contemporary Organic Chemistry.* 2nd ed. Saunders, Philadelphia, PA.

TESTER, P. A., FOWLER, P. K., AND TURNER, J. T. (1989). Gulf Stream transport of the toxic red tid dinoflagellate, *Pyychodiscus brevis* from Florida to North Carolina. In *Proceedings of Symposium o Novel Phytoplankton Bloom: Causes and Impacts of Recurrent Brown Tides and Other Unusual Blooms* Stony Brook, NY. *Cited In:* Geraci, 1989.

TEUFEL, M., BOEHM, I., AND NIESSEN, K. H. (1991). Belastung unserer kinder mit pestiziden, PCB un potentiell kritischen anionen. *Monatschr. Kinderheilkd.* **139**, 442–449.

THOMAS, P. T., AND FAITH, R. E. (1985). Adult and Perinatal Immunotoxicity Induced by Halogenate Aromatic Hydrocarbons. In *Immunotoxicity and Immunopharmacology* (Dean, Luster, Munson an Amos, eds.). Raven Press, New York.

TILLITT, D. E., ANKLEY, G. T., GIESY, J. P., LUDWIG, J. P., KURITA-MATSUBA, H., WESELOH, D. V., ROSS P. S., BISHOP, C. A., SILEO, L., STROMBORG, K. L., LARSON, J., AND KUBIAK, T. J. (1992). Polychlorinatec biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. *Environ. Toxicol Chem.* **11**, 1281–1288.

TILLITT, D. E., GIESY, J. P., AND ANKLEY, G. T. (1991). Characterization of the H4IIE rat hepatoma cel bioassay as a tool for assessing toxic potency of planar halogenated hydrocarbons in environmental samples *Environ. Sci. Technol.* **25**(1), 87–92.

TILSON, H. A., JACOBSON, J. L., AND ROGAN, W. J. (1990). Polychlorinated biphenyls and the developing nervous system: Cross-species comparisons. *Neurotoxicol. Teratol.* **12**, 239–248.

TRAVIS, C. C., AND HATTEMER-FREY, H. A. (1989). A perspective on dioxin emissions from municipal solid waste incinerators. *Risk Anal.* **9**(1), 91–97.

UNGER, M., KIAER, H., BLICHERT-TOFT, M., OLSEN, J., AND CLAUSEN, J. (1984). Organochlorine compounds in human breast fat from deceased with and without breast cancer and in a biopsy material from newly diagnosed patients undergoing breast surgery. *Environ. Res.* **34**, 24–28.

UNGER, M., AND OLSEN, J. (1980). Organochlorine compounds in the adipose tissue of deceased people with and without cancer. *Environ. Res.* **23**, 257–263.

UNGER, M., OLSEN, J., AND CLAUSEN, J. (1982). Organochlorine compounds in the adipose tissue of deceased persons with and without cancer: A statistical survey of some potential confounders. *Environ. Res.* **29**, 371–376.

VALVANI, S. C., YALKOWSKY, S. H., AND ROSEMAN, T. J. (1981). Solubility and partitioning. IV. Aqueous solubility and octanol–water partition coefficients of liquid nonelectrolytes. *J. Pharmacol. Sci.* **70**(5), 502–550.

VAMVAKAS, S., BITTNER, D., AND KOSTER, U. (1993). Enhanced expression of the protooncogenes c-myc and c-fos in normal and malignant renal growth. *Toxicol. Lett.* **67**, 161–172.

VAN DEN BERG, M., CRAANE, B. L., SINNIGE, T., LUTKE-SCHIPHOLT, I., SPENKELINK, B., AND BROUWER, A. (1992). The use of biochemical parameters in comparative toxicological studies with the cormorant (*Phalacrocorax carbo*) in the Netherlands. *Chemosphere* **25**, 1265–1270.

VAN DER KRAAK, G., MCMASTER, M. E., AND MUNKITTRICK, K. R. (1992). *Alterations in Reproductive Function in Fish as an Indicator of Exposure to Pulp Mill Effluents.* Presented at the 7th Colloquium on Pulp and Paper Mill Effluents. December 15 & 16, 1992. Pulp and Paper Centre, University of Toronto, Toronto, Ontario, Canada.

VAN DER WEIDEN, M. E. J., VAN DER KOLK, J., PENNINKS, A., SEINEN, W., AND VAN DEN BERG, M. (1990). A dose–response study of 2,3,7,8-TCDD in the rainbow trout (*Salmo gairdneri*). *Mar. Environ. Res.* **28**, 509–513.

VERSCHUEREN, K. (1983). *Handbook of Environmental Data on Organic Chemicals.* 2nd ed. Van Nostrand Reinhold, New York.

WARDELL, R. E., SEEGMILLER, R. E., AND BRADSHAW, W. S. (1982). Induction of prenatal toxicity in the rat by diethylstilbestrol, zeranon, 3,4,3',4'-tetrachlorobiphenyl, cadmium and lead. *Teratology* **26**, 229–237.

WEBER, H., HARRIS, M. W., HASEMAN, J. K., AND BIRNBAUM, L. S. (1985). Teratogenic potency of TCDD, TCDF and TCDD-TCDF combinations in C57BL/6N mice (cleft palate; toxic interactions, additivity). *Toxicol. Lett.* **26**, 159–167.

WESTER, P. G., AND VAN DER VALK, F. (1990). Tetrachlorobenzyltoluenes in eel from the Netherlands. *Bull. Environ. Contam. Toxicol.* **45**, 69–73.

WILLIAMS, L. L., AND GIESY, J. P. (1992). Relationships among concentrations of individual polychlorinated biphenyl (PCB) congeners, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TCDD-TEQ), and rearing

mortality of chinook salmon (*Oncorhynchus tshawytscha*) eggs from Lake Michigan. *J. Great Lakes Res.* **18**(1), 108–124.

WILLIAMSON, D. G., AND CVETANOVIC, R. J. (1968). Rates of reaction of ozone with chlorinated and conjugated olefins. *J. Am. Chem. Soc.* 90(16), 4248–4252.

WILLIAMSON, G. M., AND WEISBURGER, J. H. (1991). Chemical Carcinogenesis. In *Toxicology: The Basic Science of Poisons.* (M. O. Amdur, J. Doull, and C. D. Klaassen, eds.). pp. 127–200. Pergamon Press, New York.

WOO, Y.-T., LAI, D. Y., ARCOS, J. C., AND ARGUS, M. F. (eds.). (1985). *Aliphatic and Polyhalogenated Carcinogens,* Vol. 3B. In *Chemical Induction of Cancer. Structural Bases and Biological Mechanisms.* Academic Press, San Diego, CA.

YAMASHITA, N., TANABE, S., LUDWIG, J. P., KURITA, H., LUDWIG, M. E., AND TATSUKAWA, R. (1993). Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. *Environ. Pollut.* **79**, 163–173.

ZOETEMAN, B. C. J., HARMSEN, K., LINDERS, J. B. H. J., MORRA, C. F. H., AND SLOOF, .W. (1980). Persistent organic pollutants in river water and groundwater of the Netherlands. *Chemosphere* 9, 231–249.