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POTENTIAL AREAS OF ENDOCRINE DISRUPTOR EXPOSURE RESEARCH: FROM SOURCE TO POTENTIAL DOSE

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INTRODUCTION

Since the late 1980's, studies have postulated linkages between exposures to anthropogenic and natural chemical substances and hormonal dysfunction in mammalian and lower level species. These so-called "endocrine disruptors" are exogenous agents that can interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones in humans and wildlife, and are responsible for maintaining homeostasis and regulating animals' reproduction and development (Kavlock, *et al.*, 1996). Presently, the U.S. Environmental Protection Agency (EPA) is considering the role of the research community in identifying and reducing the risks and exposures of these substances in the environment.

This paper highlights important areas of endocrine disruptor exposure research and the manner in which this research can fit within the Agency's risk paradigm, particularly as it relates to human and ecosystem exposure. To illustrate the importance of these research areas, two fungicides that are suspected of eliciting endocrine responses are discussed.

A wide range of chemical compounds are suspected of eliciting these effects. Endocrine disruptors include organohalogen, organometallic, organophosphate, and carbamate pesticides; antibiotics; natural and synthetic hormones; pyrethroids; polychlorinated biphenyls; and combustion byproducts, including polycyclic aromatic hydrocarbons, halogenated dioxins, and halogenated furans. They may act as agonists that directly bind to estrogen-, androgen-, and progesterone-receptors in the cell, or as antagonists that interfere with normal binding at the membrane sites. Some compounds may be neither direct agonists nor antagonists, but act indirectly on the endocrine system by inducing changes in the reproductive, immune, or nervous system activity that, in turn, elicit endogenous responses in the endocrine system (Gray *et al.*, 1995; Colborn, *et al.*, 1993).

Gray, *et al.* (1996) have identified a number of fish, bird and mammal studies that link exposure to certain chemical compounds to demasculinization and feminization of males and defeminization and masculinization of females. A well publicized incidence of a suspected ecological link between chemical exposure and endocrine disruption has been observed in male alligators in Lake Apopka, Florida. These alligators exhibited reproductive and hormonal abnormalities; *e.g.*, elevated levels of estrogen, abnormal seminal vesicles, and smaller than normal penises, after an exposure due to a large spill of dicofol (containing 15% DDT).

Endocrine disruptors pose several challenges to exposure assessment, in part due to the many chemical classes that have been implicated. The pathways between sources and exposure can be complex (Vallero, 1996a). In addition to typical lifetime dose-response relationships, endocrine disruptor exposure estimates require information about the timing of exposures. Linking exposures and biological responses is complicated by the relatively brief critical periods of susceptibility during an organism's development, during endocrine regulation of metabolism and homeostasis, and during susceptible reproduction "windows" (e.g., estrus, early fetal development) at various times in the organism's life span. Unfortunately, reliable laboratory and

field methods are not available for several of the suspected endocrine disruptors currently under review by the environmental scientific community. An understanding of the modes of toxicological action, measurements of the concentrations of these substances in the various environmental media, and improved means of modeling their movement and transformation among environmental media are fundamental to conducting human and ecosystem risk assessments.

Improved methods and models are needed to measure and to predict the exposure to these substances. A modicum of understanding of exposure is also needed to design studies related to endocrine disruption; *e.g.*, studies of biological mechanisms and effects should address chemical species of endocrine disruptors that are most prevalent in the environment. The consensus emerging from the scientific debate surrounding endocrine disruptors is that there are insufficient data to resolve objectively the relative ecological and human health risks associated with these environmental contaminants.

The suspected endocrine disruptors that have been studied are predominantly organic compounds or organic forms of heavy metals that are persistent, can bioaccumulate, and can biomagnify in the food chain. Subtle variations in chemical form and physicochemical characteristics (*e.g.*, planarity, isomerization, equilibria, and sorption affinities), may manifest themselves in numerous ways that may affect exposure (*e.g.*, differences in transport and routes of exposure, increased or decreased bioavailability, changes in exposure pathways, potential for atmospheric and hydrological transformation, and fate). Most polychlorinated biphenyls, for example, would be expected to have more affinity for the sediment than for the water, since they are relatively hydrophobic. Risk analysts and exposure researchers must understand complex exposure patterns, rather than net annual exposure estimates. Developmental biology dictates that certain exposure windows of vulnerability can be expected to follow temporal and seasonal patterns of endocrine functions.

A RISK ASSESSMENT PERSPECTIVE

A national endocrine disruptor research program should follow EPA's risk assessment framework, and explore methods and models to estimate and to predict exposure to these substances. The exposure component of this research plan should follow the steps shown in Figure 1. Calculations of exposure are a function of dose. The U.S. Environmental Protection Agency (1992) defines four types of dose: potential dose (D_P); applied dose (D_A); internal dose (D_I); delivered dose (D_D); and biologically effective dose (D_{BE}). The exposure pathways begins with an organism's first contact with a substance (D_P) to its intake, absorption, and metabolism (D_A, D_I and D_D) to its effect on the target organ (D_{BE}).

Measurements of D_P can often provide a reasonable estimate of exposure; *i.e.*, the concentration of a contaminant around an organism. For airborne contaminants, D_A is a function of concentration, time, and ventilation. It is difficult or impossible to measure D_{BE} directly, so D_A, D_I and D_P are most often expressed by biomarkers, *i.e.*, "indicators of changes or events in human biological systems" (1991). Biomarkers may either be the contaminant itself or metabolites indicating exposure to the contaminant; *e.g.*, increased concentration of cotinine (a metabolite of nicotine) in blood resulting from exposure to tobacco smoke. Similarly, biomarkers in ecosystems are "biochemical, physiological, or histological indicators of either exposure to or effects of xenobiotic chemicals at the suborganismal or organismal level" (Huggett, *et al.*, 1992). Biomarkers can also apply to ecological exposure, although they are not often classified as measures of dose (ecologists may apply the terms, "biotic and abiotic accumulation"). For example, Hunsaker *et al.* (1990) have suggested measuring cholinesterase levels and porphyrin accumulation to indicate the level of ecosystem exposure.

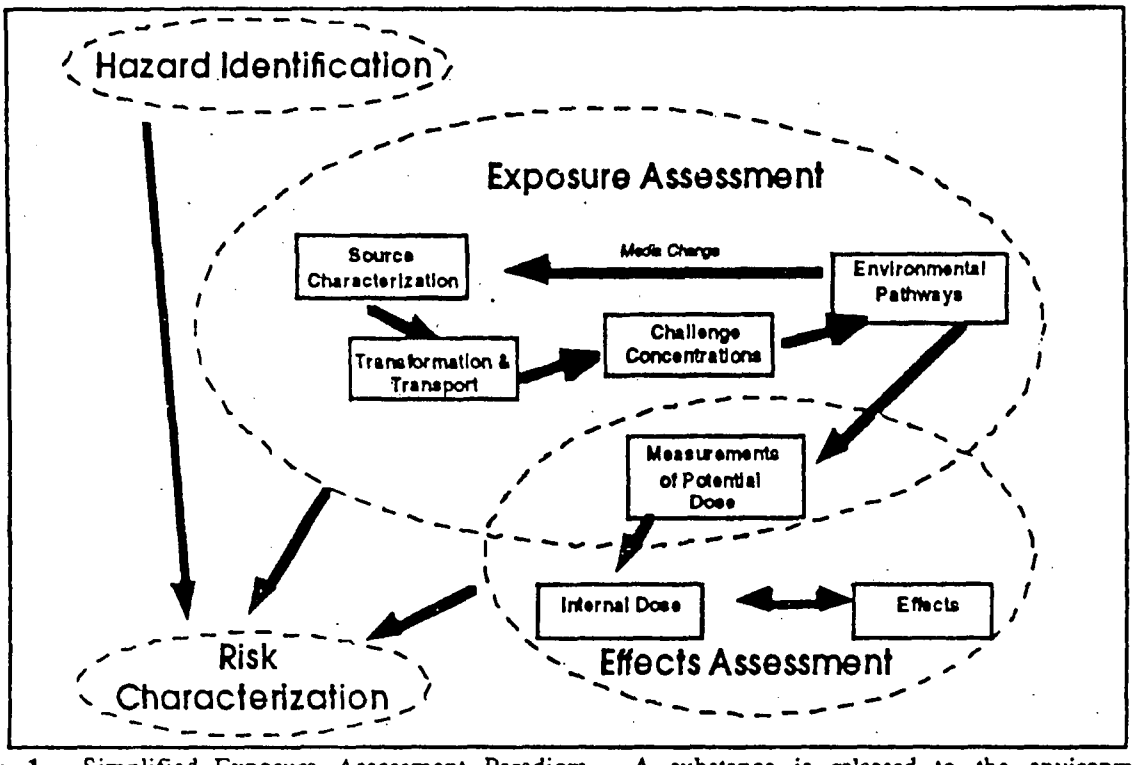


Figure 1 Simplified Exposure Assessment Paradigm. A substance is released to the environment, is transported, may be transformed chemically and physically, and can move through various pathways; *e.g.*, water, air, soil and sediment. After a substance reaches an environmental pathway; *i.e.*, media change, this can be tantamount to being a new source, in essence starting the process again from source characterization. The fate is then determined iteratively via mass balance.

Methods for estimating ecosystem exposure can be similar to those for human exposure assessment, as indicated in Figure 2, but the methods may differ in important ways. Both ecosystem and human exposure assessments are often concerned with sensitive subpopulations, many pollutants are both human and ecological stressors, and ambient measurements for some pollutants can be indicators of both human and ecosystem exposure (*e.g.*, ozone). Human risk assessments provide an expression of the likelihood that an adverse outcome will result from a given hazard; *e.g.*, 10^{-6} chance of cervical cancer in a population exposed to a particular pollutant. Ecological risk assessments are also expressions of the likelihood of an adverse outcome, but the expression depends upon the "environmental value" of concern; *e.g.*, biological diversity, sustainability, and aesthetics (Environmental Monitoring and Assessment Program, 1993). A major difference between human and ecological exposure paradigms is the level of biological organization at which contaminants are typically studied; *i.e.*, human epidemiology considers population exposure for one species (human) and medical research considers responses at various doses and exposures for an individual human being. Ecological exposure assessments often attempt to address substances that affect the whole ecosystem. These may include exposures to a community (several species), as evidenced by contaminant concentrations in certain indicator organisms or "sentinel" species. Ecosystem exposure also considers population exposures for a target species (e.g., top predator tissue concentrations of a contaminant suspected of reducing fecundity). Ecosystem exposures can even be extrapolated from measurements of abiotic media; e.g., an estimate of fish community exposure extrapolated from water column concentrations of a contaminant.

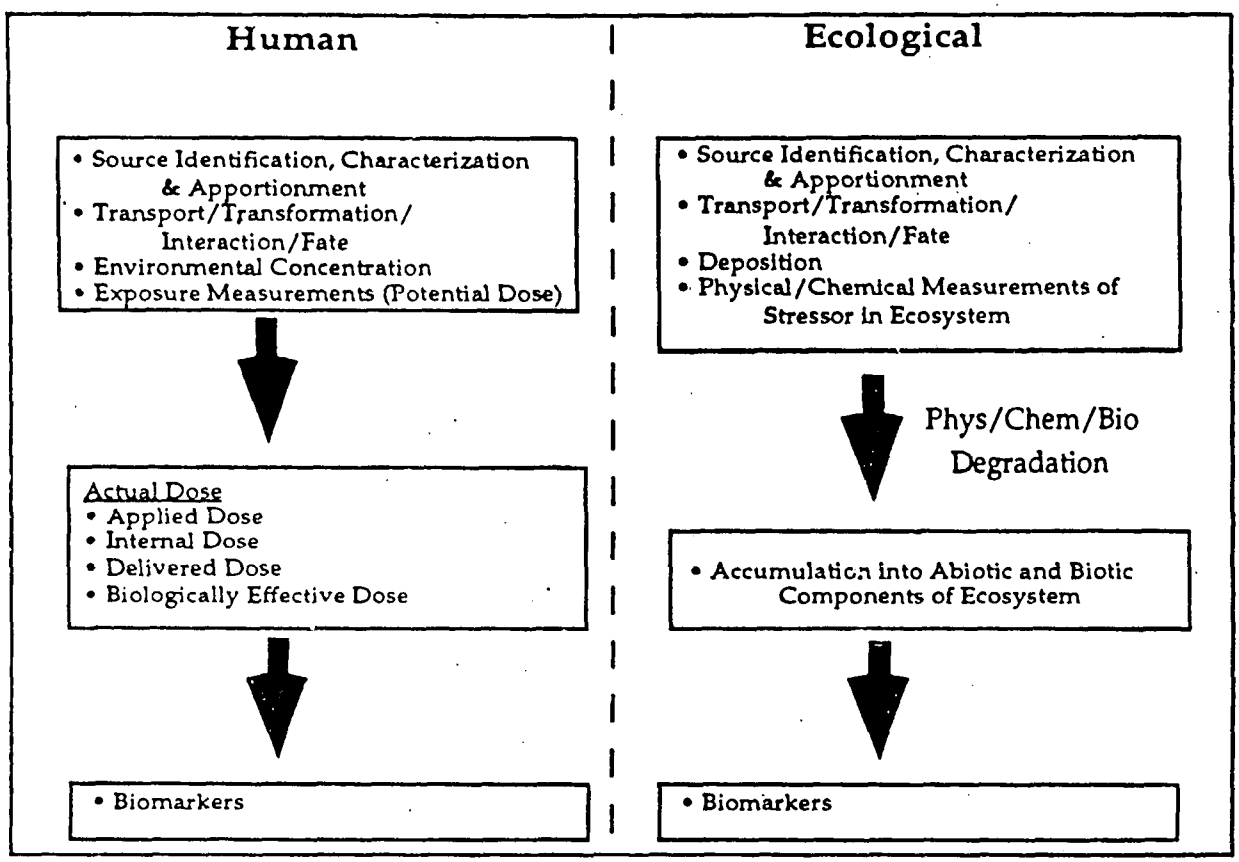


Figure 2 Exposure components of risk paradigms are similar for humans and ecosystems (Vallero, 1996a).

Human exposure can be expressed as the lifetime average daily dose (LADD). Each route of exposure must be considered; *i.e.*, ingestion (water, food, and soil), inhalation of gases and particles, and dermal exposures. Based upon Derelanko's (1995) expressions of LADD, total LADD may be calculated as the sum of all LADD values via all routes:

$$LADD_T = LADD_A + LADD_I + LADD_D \quad (1)$$

where: $LADD_T$ = lifetime average daily dose (mg/kg/d) via all routes
$LADD_A$ = lifetime average daily dose (mg/kg/d) via inhalation
$LADD_I$ = lifetime average daily dose (mg/kg/d) via ingestion
$LADD_D$ = lifetime average daily dose (mg/kg/d) via dermal routes.

Further, each route can be further, subdivided. For example, $LADD_T = LADD_g + LADD_p$

$$LADD_g = \frac{(C)(IR)(EL)(AF)(ED)}{(BW)(TL)} \quad (2)$$

where: $LADD_g$ = lifetime average daily dose (mg/kg/d) from inhaling vapors;
C = concentration in air (mg/m^3);
IR = inhalation rate (m^3 /h);
EL = exposure length (h/d);
AF = uptake or absorption factor (dimensionless, fraction of inhaled C absorbed);
ED = duration of exposure (d);
BW = body wt (kg);
TL = typical lifetime (d)

$$LADD_p = \frac{(C_p)(PC)(IR)(RF)(EL)(AF)(ED)(10^{-6})}{(BW)(TL)} \quad (3)$$

where: $LADD_p$ = lifetime average daily dose (mg/kg/d) from inhaling particle matter (liquid and solid);

C_p = concentration of contaminant sorbed on or in particle (mg/m^3);

PC = particle concentration in air (mg/m^3);

10^{-6} = converts kg to mg.

Equations 2 and 3 also indicate that exposure models should incorporate physicochemical properties associated with transport, transformation and fate in air, soil, water, and sediment transport capabilities of existing compartmental models. For example, Figure 3 illustrates three idealized bimodal distributions for particles. Such distributions can provide weight-of-evidence for

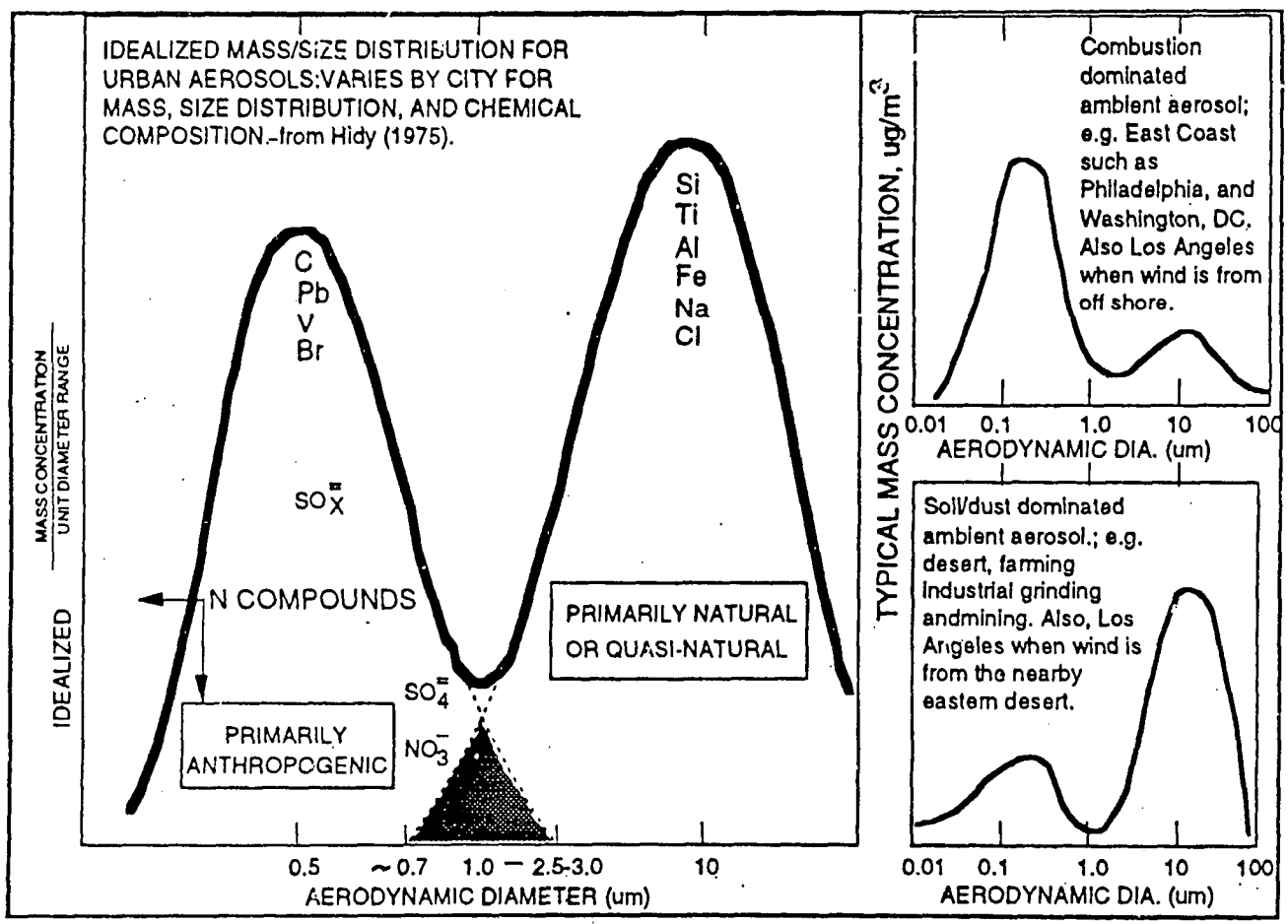


Figure 3 Particles often display a bimodal distribution by mass, originate from multiple sources, show dynamic growth and reactivity, and are carriers of other pollutants (Hidy, 1975). The upper right mass distribution is typical for an area dominated by anthropogenic (combustion) sources, while the bottom distribution is typical for areas where particles are generated from noncombustion sources (*e.g.*, re-entrained soil and mining activities).

anthropogenic or natural sources of contaminants. Karickhoff and Long (1996) have developed the SPARC model which characterizes the potential environmental fate of substances based upon vapor pressure, lipophilicity (*e.g.*, K_{ow}), activity coefficients, water solubility, phase partitioning

(*i.e.*, Henry's Constant) and ionization potential (pK_a). Therefore, physical and chemical characteristics, such as phase distribution and a substance's affinity to accumulate in various environmental compartments, can profoundly affect the estimates of exposure to humans and wildlife.

Exposure assessments should be conducted at appropriate spatial and temporal scales, depending upon the hypothesis or research question being investigated. Timing is complicated by triggering and response mechanisms in the endocrine system at certain stages of development in humans and wildlife. These windows of exposure, where the organism is particularly vulnerable to hormonal dysfunction, must be addressed in any exposure calculations. The author recommends:

$$CWDD_{EDC} = (LADD_T + CW)(SF)(MT) \quad (4)$$

where: $CWDD_{EDC}$ = Total critical window endocrine disruptor exposure (mg/kg/d);
CW = Additive dose during critical windows of vulnerability (mg/kg/d);
SF = sensitivity factors; *e.g.*, demographics for human populations, species sensitivities for wildlife (dimensionless);
MT = maternal transfer and transgenerational multiplier (dimensionless).

POTENTIAL ENDOCRINE DISRUPTOR EXPOSURE RESEARCH AREAS

The National Academy of Sciences (1991) has recommend approaches for assessing human exposure to airborne pollutants (Figure 4), emphasizing the need for data from direct measurements (personal and biomarker monitoring) and from indirect approaches (especially to gain knowledge about activities). At the outset, however, exposure research for endocrine disruptors should emphasize the physicochemical characterization of known or highly suspect endocrine disruptors. As indicated, even slight differences in physicochemical properties can greatly affect environmental fate; therefore exposure estimates must begin with an understanding of how these substances can be expected to behave in the various media and their fate. The development and adaptation of compartmental transport and fate models can be a major focus of this research.

Characterizing Chemodynamic Fate

Chemical substances reside and move through environmental "compartments;" *i.e.*, air, soil, water, sediment, and biota, being transported and transformed before reaching the sites of their ultimate fate. Such compartments can be simulated in mathematical models to predict the persistence. bioaccumulation, bioconcentration, and biomagnification of these substances within each medium, according to physicochemical properties such as vapor pressure, water/lipid solubility, bioaccumulation factors, and chemical half-life. Modeling can apply to both human and ecosystem exposures; such as food chain models, however, compartmental exposure models have principally been used to address outcomes other than endocrine disruption. A number of researchers, including Cohen and Clay (1994), have developed models to simulate the movement and change of chemical substances in the environment, as a function of their physicochemical characteristics. These properties dictate the potential sorption, transformation, transfer, and fate in soil, sediment, water and air and uptake by biota; the ability of substances to enter the food chain; and the magnification of chemical concentrations at higher trophic levels for those substances that accumulate.

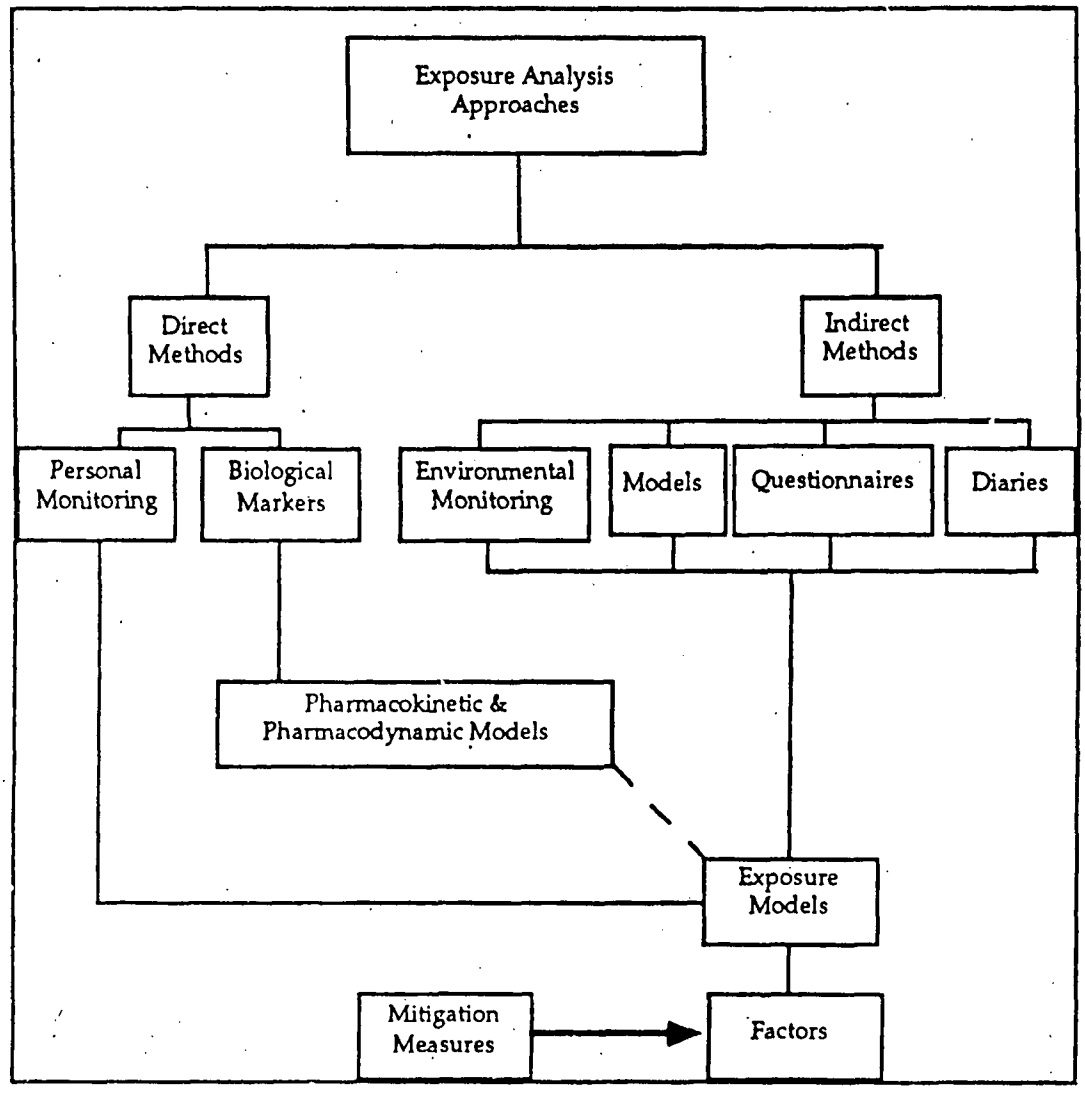


Figure 4 Possible approaches for analysis of air contaminant (From: National Academy of Sciences, 1991). The dashed line between pharmacokinetic and pharmacodynamic models and exposure models have been added by author to show that exposure models can be derived from direct measurement data, from routines from other models, and from combinations of measured and derived data.

In sediments, for example, transformation pathways and kinetics are determined by a complex interaction of microbial, chemical, and physical processes. The interplay of these processes should be an overarching theme for any study of chemical fate in the environment. Many of the suspect endocrine disrupting chemical substances identified to date are low solubility, neutral organic compounds that are highly sorbed on the organic carbon phases of sediments. Currently available predictive tools are based on hydrophobic solution theory, and are reliable for estimating the magnitude of sorption of such compounds on sediments. Comparable tools for estimating the kinetics of the sorption and desorption processes are lacking. Work is also needed to develop models for predicting the sorption of endocrine disruptors to particles in sediment, soil, water, and air, under varying environmental conditions (e.g., pH, moisture, organic matter types and concentrations, ionic strength, and concentrations, shapes and sizes of particles).

Providing Improved Exposure Data

Reliable and standardized data bases are vital in testing effects/exposure hypotheses, and in evaluating exposure and effects models. A strategic approach for using or modifying existing

monitoring programs to assess current and historical effects of endocrine disruptors should be developed. Federal and other data bases need to be reviewed for reliability (meta-data, quality assurance, documentation, frequency, and methods), and an assessment be given to the scientific community regarding the data quality and the means for accessing these data (electronically and manually). Several existing monitoring programs that collect data could be used to help in problem formulations for risk assessments, or to support exposure or effect characterizations in retrospective risk assessments. Examples in the U.S. include the Environmental Monitoring and Assessment Program (EMAP) of the EPA, the National Status and Trends Program (BEST) of the Department of the Interior's National Biological Service, the National Water Quality Assessment Program (NAWQA) administered by the U.S. Geological Survey, and a variety of state and joint international monitoring programs.

Identifying Major Knowledge Gaps and Uncertainties

The ranges of uncertainty must be identified and incorporated into exposure models. Improved toxicokinetic and structure activity models need to be linked with the physicochemical characteristics of suspect endocrine disruptors, especially at critical and sensitive early life-stages. Compartmental models and laboratory studies must be linked to field research by developing mechanism-based dose response models. Exposure levels observed in the field will be used as a basis for identifying realistic dose ranges in laboratory experiments.

Exposure scientists will need specific and sensitive biomarkers. Effects and exposure biomarkers must be calibrated to adverse individual- and population-level effects. Biomarkers of exposure are an essential adjunct to environmental measurements in developing and verifying human and ecosystem exposure models. They are also needed to screen ecosystems for exposures and to improve exposure estimates in future epidemiological studies. Field evaluations of these markers should establish which are most predictive of population-level effects (*i.e.*, which are most useful for establishing cause and effect relationships). This necessitates the evaluation of "normal" values and the uncertainty associated with their measurement.

Research at Appropriate Spatial, Biologic, and Temporal Scales

Endocrine disruptor research will take place at spatial scales ranging from subcellular exposure to regional. Methods for assessing exposure for an individual organism (*e.g.*, one human being) differ from methods used to assess population exposure. Likewise, estimating exposures for a single ecosystem component; *e.g.*, a lake or wetland, will be different from a large-scale exposure assessment of region or biome. Geographic scale also plays a crucial role in model selection. There may be a need for predictive capability on the micro-scale (*e.g.*, occupational, residential), field-scale (*e.g.*, production plant emissions impacting adjacent ecosystems or human populations), regional scale (*e.g.*, farm applications and resulting human and ecosystem exposures in an entire watershed) and global-scale (*e.g.*, long-range transport and exposure at remote sites).

At lower levels of biological organization, a researcher may be able to determine signals of exposure for a wide array of contaminants, and provide detailed and specific information about a subject's activity patterns. Often, however, scientists are asked to estimate exposure of entire populations or target groups, wherein gathering detailed and specific information about the exposure of each individual in a population is scientifically and economically infeasible. Moreover, in the case of ecosystems, detailed information about individuals may have less importance than the interrelationships and diversity of a larger ecological community; true to the adage, "not seeing the forest for the trees." The hypothesis or study objective determines the scale of an exposure assessment.

The temporal scale can range from very short duration, single event to random episodic events to long-term, discrete exposures (e.g. annual or seasonal) to continuous exposures. The temporality of the exposure must be considered in the study design. For example, an episodic event may require monitoring until the chemical and biological indicators regain equilibrium, and long-term, continuous exposure studies may call for seasonal and annual time series and status and trend assessments. Even episodic exposures may require lengthy follow-up studies, however, since the exposures may be transgenerational, and successive generations become exposed via maternal transfer.

Exposure Hypothesis Testing

Comprehensive investigations of a small number of experimental sites/systems with problems that are known or strongly-suspected to be related to endocrine disruptors could yield valuable information about human and wildlife exposures. Such an integrated study, conducted at multiple levels of biological organization, with both laboratory and field components, may provide insights into the identification of sensitive measurement endpoints and species, and extrapolation among endpoints, species and chemicals.

Both biological and exposure measurements need to be collected for areas expected to have elevated concentrations of suspected endocrine disruptors in various environmental media. Biomarker researchers can test screening tools, *in situ* results can be compared to *in vivo* and *in vitro* findings, and biologically plausible hypotheses linking exposure and effects can be tested. At the outset, using professional judgment, scientists could select pilot study sites based upon weight-of-evidence that populations have been affected by exposure to endocrine disruptors. Such evidence could be derived from ecological epidemiology, exposure screens, historical data suggesting a likelihood that endocrine disruptors are present in one or more environmental media, or suggestions from source or fate models of a "hot spot." For ecosystems, pilot studies should address direct and indirect effects of endocrine disruption in multiple phylogenetic groups and trophic levels.

An emerging area of concern is the impact of endocrine disruptors on mammalian immune systems. Some agricultural chemicals, such as DDT and its metabolites, can act as both endocrine disruptors and immunosuppressants. The initial mode of action is to suppress adrenal secretions which, in turn, directly and indirectly decreases the immune response to bacterial infection. A possible exposure study may include measurements of mammalian serum antibody titers. Some veterinary pharmaceuticals and antibiotics are also administered to promote growth in livestock, which may select out the more resistant strains of enteric bacteria, the so-called "super bugs." The fate and transport in the various environmental media of these multi-active compounds should be examined. Various species of bacteria at contaminated sites should be analyzed for increased resistance to selected antibiotics. An important exposure research question is whether the bacteria are being transported among environmental compartments, thus spreading resistance to unexposed bacterial populations. Identifiable bacteria strains may also prove useful as exposure biomarkers for specific endocrine disrupting agricultural pharmaceuticals.

Studies will be needed to test and confirm results from predictive, integrated models that incorporate structure-activity relationships, toxicokinetics, bioenergetics, environmental chemistry, and population ecology. They can provide a means for testing effects and exposure screening tools, and would provide multimedia samples for analytical methods development. Although these pilots would likely focus upon ecosystem level effects and endocrine disruptor concentrations in environmental media, they may also present the opportunity to conduct human residential studies compare expected exposures from human exposure models to actual exposures under actual environmental conditions where weight-of-evidence suggests a human biological response; *e.g.* concentrations in carpet, food, indoor and outdoor air, and drinking water at a small number of sites around a former facility where a suspect endocrine disrupting substance was manufactured.

In addition to measurements of endocrine disruptors in highly contaminated areas, some estimate of variability of contamination in different regions may be obtained via monitoring sites where air, water, soil, sediment, and vegetation samples can be gathered. These samples can provide valuable information about areas other than the highly contaminated areas that will be addressed in the pilot studies. The National Exposure Research Laboratory, for example, is establishing such a site near each of its laboratory facilities in Georgia, Nevada, North Carolina, and Ohio. At a minimum, these "near-laboratory" sites will provide a means for developing and testing measurement protocols for a wide range of pollutants, including suspected endocrine disruptors.

FUNGICIDES IN SOIL: A POTENTIAL AREA OF ENDOCRINE DISRUPTOR CHEMODYNAMICS RESEARCH

Agricultural operations have long used many neurologically active pesticides that have subsequently been shown to effect the endocrine systems of animals. Several suspect endocrine disruptors presently under review by the U.S. EPA (1996) are agricultural chemicals or their decay products; *e.g.*, DDT and DDE, that are relatively lipophilic and tend to bioaccumulate in the environment. Recently, in response to concerns about groundwater contamination, pesticide manufacturers have reformulated pesticides to dissipate upwardly to the atmosphere to prevent downward migration. These changes in chemodynamics accentuate the importance of advancing the understanding of soil-to-air fluxes.

Several fungicides are formulated with active organochlorine and organometallic functional groups (Meister, 1996), often making them persistent and semi-volatile substances (vapor pressure = 10^{-2} to 10^{-8} kilopascals). Semivolatile compounds do not readily dissipate and can remain active for longer periods of time than volatile compounds (Lewis and Gordon, 1996). Unlike nonvolatiles, after being incorporated into the soil, semivolatiles may later be transported from soil to air. This flux rate is diffusion controlled, and is proportional to vapor pressure. In the atmosphere, they may remain as gaseous pollutants or may be sorbed to particles that can travel long distances and later concentrate in various environmental media and biotic tissue.

Within soil and sediment, sorption and degradation processes exert the largest controls over the fate of agricultural chemicals in the environment. Adsorption to soil particles decreases the vapor pressures of pesticides, and is dependent upon soil conditions. The rate of evaporation from the soil column also affects xenobiotic flux. Plant uptake rates of dissolved chemicals and transport to the ground and surface waters are controlled by the chemicals' physical and chemical properties and the conditions of the soil (Rao, *et al.* 1993). Spencer and Cliath (1990) identified soil water content, physical and chemical properties, concentration of the compound, and soil properties, especially soil organic matter, as the most important factors controlling adsorption rates (Nash and Hill, 1990). Fungicide half-life in the soil and sediment, degradation product formation, and kinetics in the soil matrix and air column and in the sediment and water column must be characterized properly before reliable endocrine risk assessments are possible. Characteristics of the compounds affect its potential for transport and transformation, such as water solubility, lipophilicity, dissociation, and molecular weight. Characteristics of the environmental media also determine transport and transformation, such as soil and solution pH and redox conditions, soil moisture, soil texture and structure, and type and amount of soil organic matter.

The modes of toxic actions of these substances are determined by the physicochemical characteristics. These same characteristics may influence a compound's ability to persist and to bioaccumulate, and to elicit longer term effects in humans and wildlife. Slight variations in physicochemical characteristics, such as planarity and isomerization, may drastically change soil fungicide exposure and effects.

Movement and transformation in the environment can be illustrated by two fungicide classes associated with endocrine effects in humans and wildlife; *i.e.*, dicarboximides and organotins.

Vinclozolin

Vinclozolin is a dicarboximide fungicide whose structure is shown in Figure 5. Since it has a dichlorobenzene group, vinclozolin may be classified as an organochlorine compound, the group presently most often associated with endocrine disruption. It may also be classified as a carbamate pesticide, since the right side of the structure is derived from carbamic acid. Two principal degradation products result from opening the carbamate ring: a buteonic acid (2-[[(3,5-Dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid), referred to as "M1"; and an enanilide (3',5'-Dichloro-2-hydroxy-2-methylbut-3-enanilide), called "M2". Both degradation products have been isolated in plants and soils (Kelce, *et al.*, 1994). M1 is a reversible reaction, where the carbamate ring closes and returns to the vinclozolin structure, whereas M2 is a non-reversible degradation product.

Vinclozolin is registered in the United States and Europe as a fungicide for grapes, strawberries, sunflowers, rape seed, soft fruits, hops, ornamental plants. It has been shown to alter mammalian sex differentiation by inhibiting androgen receptor activity (Kelce *et al.*, 1994). Developing fetuses are extremely sensitive to vinclozolin exposure; exposures to rat fetuses has been associated with infertility, deformed genitalia, and reduced sperm counts (Wong *et al.*, 1995; Gray, *et al.*, 1994). Kelce, *et al.* (1996) have found that it induces antiandrogenic developmental effects *in vivo* and that it inhibits androgen receptor (AR) binding and AR gene expression *in vitro*.

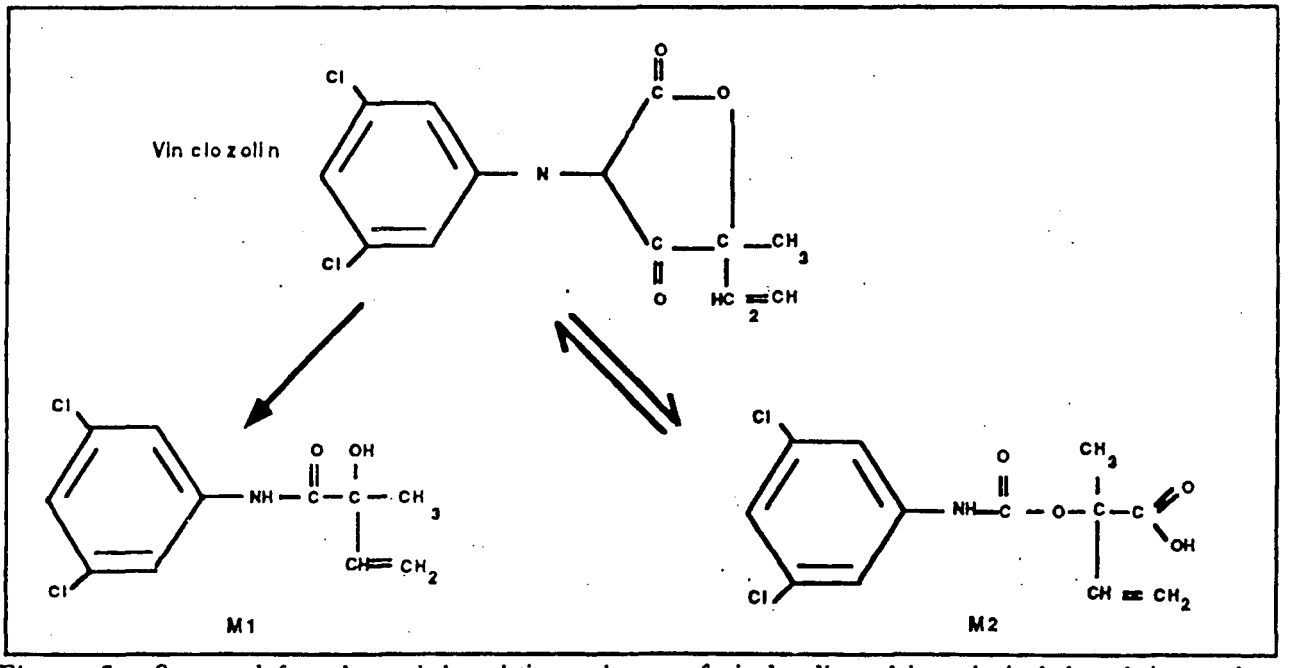


Figure 5 Structural formulae and degradation pathways of vinclozolin and its principal degradation products (from Szeto, *et al.*, 1989a).

Physical and chemical processes determine the amount and form of vinclozolin that may reach the soil. Application rates and methods affect vinclozolin degradation and persistence, since it may applied be to foliage and above-ground plant parts and migrate downward to soils, or it may be incorporated directly into soil; *e.g.*, it is used to prevent onion white rot and other fungi in bulb crops (Meister, 1996).

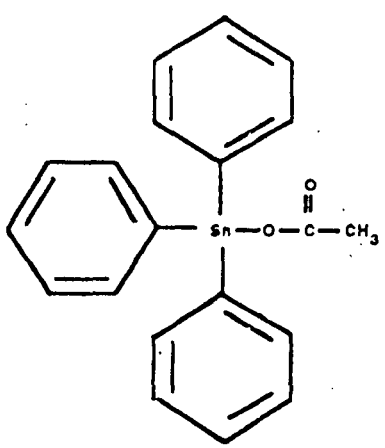
Szeto, *et al.* (1989b) found that the pH of the soil, sediment, and water column is a principal factor in vinclozolin degradation rates. The degradation is quite rapid at higher pH, and much slower at low pH. At 35 ° C, the half-life at pH 8.3 is less than one hour, and at pH 4.5 is 530 hours. This difference can be explained in part to vinclozolin's increased resistance to hydrolysis at lower pH. The pH also determines the principal degradation pathway that vinclozolin will take, with higher pH yielding more M1 and lower pH yielding more M2. A third degradation product, 3,5-dichloroaniline, has been detected after considerable time (672.3 h at pH 6.5, 1537 h at pH 5.5, and 505.8 h at pH 4.5). This points to important considerations for estimating the fate of vinclozolin. Not only does increased soil and solution acidity increase vinclozolin's persistence, but acidity also influences the degradation pathways and the appearance of secondary degradation products.

The type of application solvent also influences vinclozolin degradation and bioavailability of vinclozolin. Szeto, *et al* (1989c) compared the persistence of vinclozolin by applying the fungicide in water and acetone solutions to young pea plants and analyzing the concentration of vinclozolin in leaflets. The acetone-vinclozolin solution was significantly more persistent than the water solution. This is likely the result of the dichorophenyl group's influence on the lipophilicity of vinclozolin. The persistence observed in the field was lower than in the laboratory studies, likely to do increased photodegradation and greater moisture gradients. However, these findings indicate the importance of the original application solutions and suspensions when estimating the ultimate fate of vinclozolin. They also indicate the importance of available moisture in the soil column, which could serve to dilute the lipophilic application solvents over time.

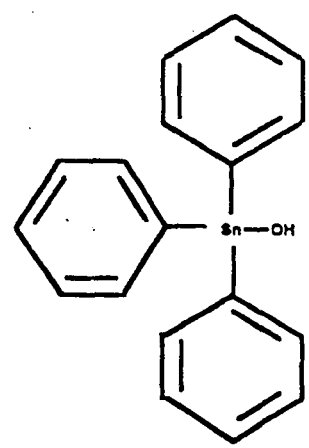
The amount and degradation pathways in the atmosphere and fluxes from soil to air are potentially important research areas. Since vinclozolin's vapor pressure is 1.6 $x10^{-5}$ kilopascals (kPa) at 20° C, which is considered semivolatile, the atmospheric transport could be an important exposure pathway. The volatility of its degradation products should also be considered in these studies.

Organotin Fungicides

Organometals are an important group of endocrine disruptors. These are compounds with covalent bonds between a carbon (C) and metal atom (Pelletier, 1995). Organotins have been associated with endocrine dysfunction (Brüschweiler, *et al.*, 1996; Ochlmann, *et al.*, 1993). Their structures are shown in Figure 6.



Triphenyltin Acetate



Triphenyltin Hydroxide

Figure 6 Structural formulae of triphenyltin acetate and triphenyltin hydroxide.

Agricultural fungicide and scab biocide applications comprise the principal source of the triphenyltins. Triphenyltin hydroxide is directly applied to soil and plant tissue as a fungicide and is used to treat seeds, root, and tubers. Triphenyltin hydroxide is also the degradation product of triphenyltin acetate, a fungicide for potatoes, rice, and sugar beets, and scab biocides for peanuts (Thayer, 1974). Other tin (Sn) compounds are used as fungicides and antifouling agents, most notably tributyltin; but their usage has been greatly restricted in the past decade.

The mechanisms of toxic action for organotins are more diverse than those of vinclozolin. They include cytotoxicity in the liver, disturbance of calcium homeostasis and induction of apoptosis in thymocytes, inhibition of ATP-synthesis and mitochondrial oxidative phosphorylation, inhibition and uncoupling of chloroplasts, ion pump inhibition and cell membrane damage, Cytochrome P450 inhibition, and intracellular enzyme inhibition (Fent, 1996). The principal endocrine endpoint observed to date is increased imposexual response in gastropods. Mud snails and other gastropods exposed to tributyltin compounds exhibit an increased incidence of pseudohermaphroditism (Bryan, et al., 1989 and Smith, 1981). Like mercury, Sn is neurotoxic. The endocrine response may be the indirect result of Sn activity in neurological system, which induces a chain of endogenous responses that ultimately elicit an endocrine response.

Most Sn-related research has focused on the aquatic environment, but fate and transport in soils and the atmosphere must also be better explained. Figure 7 shows the principal degradation pathway from the less stable acetate compounds to alkylated tin species mediated by microbes, which theoretically will ultimately degrade the fungicides and metabolites to elemental Sn (Keijzer and Loch, 1995). The process would likely move in the opposite direction under aerobic, microbially mediated conditions (from oxides and elemental tins to alkylated species).

The amount of Sn species fluxing to the atmosphere is an area in need of research. The volatility varies by species. The alkyltins are more volatile than the aryltins. Methyltins, for example, are significantly more volatile than the phenyltins. Within the phenyltins, triphenyltin acetate's vapor pressure is 1.9 $x10^{-6}$ kPa at 20° C, which is considered semivolatile, but triphenyltin hydroxide is a salt that is nonvolatile. The volatility of each degradation product should also be studied.

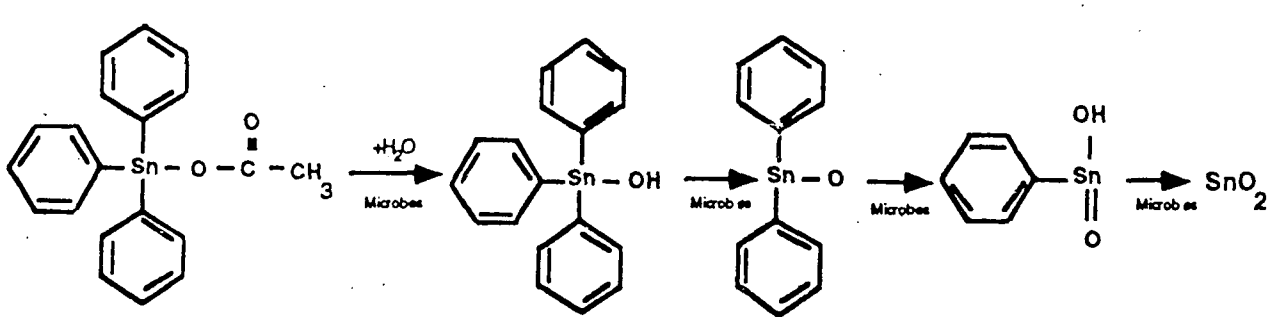


Figure 7 Degradation pathway expected for tin fungicides under aerobic soil conditions (from Keijzer and Loch, 1995).

Even the less stable organotin species can remain undegraded in soil under certain redox and acidity conditions, and if soil organic matter is plentiful. For example, triphenyltin acetate has been shown to accumulate in organic-rich upper soil horizons as a result of heavy application rates in the Netherlands (Keijzer and Loch, 1995). This phenomenon can have profound effects on the ability of certain tin compounds to migrate after deposition under certain atmospheric and aquatic conditions, but to remain in place for much longer time periods under other conditions. Environmental acidity and redox conditions can also be affected by type of organic matter present in soils and sediment. Kuballa, *et al.* (1995) found that humic substances act as methylating agents for Sn in sediment in reduced soil profiles. Therefore, anaerobic soil conditions; *e.g.*, in wetlands and rice paddies, can play a major role in Sn methylation. Little is known about the persistence of

the triphenyltin fungicides, but the persistence appears to be enhanced in reduced environments (Fent, 1996). Speciation of organotins is also pH dependent. Within normal natural ranges, higher pH values, where the hydroxides dominate, generally increase acute toxicity and uptake by organisms. However, this is mediated by the presence of organic material. Soil moisture will affect speciation of the tin compounds. For example, triphenyltin acetate is relatively hydrophilic (9 mg/l at 20° C), and is not lipophilic. Application rates will also affect soil-air fluxes by kinetic and chemical processes; *e.g.*, mass action.

In aquatic systems, increasing dissolved organic carbon reduces bioavailability by the creation of Sn-organic complexes. Therefore, organic matter in soils likely will play similar roles, especially under saturated conditions.

Accurate means of extracting, separating, and detecting the various organotin species is necessary to generate reliable estimates of exposures and risks. Analytical techniques for speciating tins are improving. Barshick, *et al*, (1996) recently found new chromatographic methods that show promise in improving the ability to speciate inorganic and organic forms of Sn from a single soil. Future research applying th· procedures to different soil types and specific Sn-related research should advance analytical capability even further.

CONCLUSIONS

The challenge of addressing endocrine disruptor risks and exposures is daunting; however, is has been shown that EPA's risk paradigm relates well to both human and ecosystem exposure assessment. Presently, some strong weight-of-evidence in isolated studies of invertebrates, fish, reptiles, birds and mammals has provided compelling reasons for linking exposures to a number of chemical compounds to endocrine disruption mechanisms in populations. These ecosystem observations suggest that similar human weight-of-evidence data bases could be valuable in directing future human endocrine disruptor exposure research.

The chemical classes represented by agricultural fungicides are sufficiently different in chemical structure and mechanisms of endocrine action to provide insights into the chemodynamic factors that are likely to influence human and ecosystem exposures. The two fungicides reviewed in the present study represent chemical groups that have been associated with *in vitro*, *in vivo*, and *in situ* endocrine effects.

In soils and sediments, transformation pathways and kinetics are determined by a complex interaction of processes that affect the amount and the degree of speciation of substances in the soil, and the potential for their release and uptake by the atmosphere, surface water, groundwater, and biota. Transport and transformation are function of characteristics of the compounds and characteristics of the environmental media. Slight variations in physicochemical characteristics may drastically change the potential for exposure and effects.

The chemodynamic behavior of fungicides in soil is basic to predicting future exposures and the efficacy of agricultural endocrine disruptor exposure prevention strategies. Improved flux measurement methods to screen and to model exposures to endocrine disrupting pesticides in various media need to be developed, validated, and incorporated into test guidelines, especially those required under Toxic Substances and Control Act and Federal Insecticide, Fungicide and Rodenticide Act.

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