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TRANSPORT, TRANSFORMATION AND FATE OF ENDOCRINE DISRUPTORS: POTENTIAL AREAS OF EXPOSURE RESEARCH

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ABSTRACT

A growing number of studies suggest that several persistent organic pollutants and some organic forms of heavy metals, such as those listed recently by the Inter-Organizational Program for the Sound Management of Chemicals, appear to mimic or to disrupt hormonal mechanisms in humans and wildlife. EPA is exploring methods and models to measure and to predict exposures to these substances. This paper addresses a number of approaches the Agency may take to conduct exposure research of endocrine disrupting compounds within the Agency's risk assessment framework.

INTRODUCTION

Studies have associated adverse health consequences in humans and wildlife species that have been exposed to environmental chemicals that interact with the endocrine system (1, 2, 3). To date, these problems have been identified primarily in human populations and animal species with relatively high exposures to organochlorine compounds, including DDT and its metabolites, polychlorinated biphenyls and dioxins, or to naturally occurring plant estrogens. Speculation has risen about environmental etiologies from several reports of declines in sperm production and reduced sperm quality in humans over the last four decades, and from reported increases in incidences of certain cancers that may have an endocrine-related basis (breast, prostate, testicular).

A critical scientific issue is whether there are sufficiently high levels of endocrine disrupting chemicals in the ambient environment to exert effects in the general population. Long-term permanent effects in the adult can result from exposure to agents during development, which can occur without apparent "birth defects" in the neonate. There appear to be critical periods for epigenetic effects on different targets. Various organs and physiological systems may be affected; e.g., reproductive, endocrine, immune, neural, behavioral, metabolic, and skeletal (4). The widespread occurrence, persistence, and magnification of endocrine disrupting chemicals in the environment and food chain make it imperative that the research primarily focus on the most critical gaps in our knowledge base. From this base, better informed regulatory and public health decisions can be made in the future.

An "environmental endocrine disruptor" has been defined as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes" (5). In addition to the so-called "environmental estrogens" and anti-androgens, the term includes agents that affect the thyroid and pituitary glands and other components of the endocrine system. Most current methods of assessing human and wildlife

health effects are targeted at detecting effects, rather than mechanisms, and may not adequately evaluate effects on the endocrine system.

To date, scientifically credible exposure assessments for endocrine disruptors have generally been lacking, but some strong weight of evidence in isolated studies of invertebrates, fish, reptiles, birds and mammals, has provided compelling reasons for the possibility that endocrine disruption mechanisms of action can be operative in populations.

Endocrine disruptors pose several challenges to exposure assessment, in part due to the heterogeneous chemical classes that have been implicated. In addition, the pathways between source and exposure are complex, and many of the disruptors are persistent and bioaccumulate. Protracted latency periods exist between the exposure and the observable manifestation of the response. Linking exposures and biological responses is further complicated by the relatively brief critical periods of susceptibility and reproduction windows during the organism's lifespan. Therefore, methods and models will be needed to measure and 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 be undertaken using 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 objectively resolve the relative ecological and human health risks associated with these environmental contaminants.

The pathways between source and exposure to endocrine disruptors are complex. Many of the suspected endocrine disruptors studied to date are organic compounds or organic forms of a few heavy metals that are persistent, can bioaccumulate, and biomagnify in the food chain. Knowledge of the nature of these factors is basic to predicting future exposures and the efficacy of exposure prevention strategies. For example, slight variations in chemical form and physico-chemical characteristics (e.g., planarity, isomerization and polarity), may manifest themselves in various 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.

Another major challenge is the need to understand complex exposure patterns, rather than the more typically calculated 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. The National Research Council (6), recognizing the importance of the developmental stage at exposure, has modified its definition of exposure assessment (change noted in *italics*): " the process of measuring or estimating the intensity, frequency, duration *and the timing of exposure*, of humans and wildlife to an agent currently present in the environment or of estimating hypothetical exposures that might arise from releases of new chemicals."

Therefore, an endocrine disruptor research program should follow the 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.

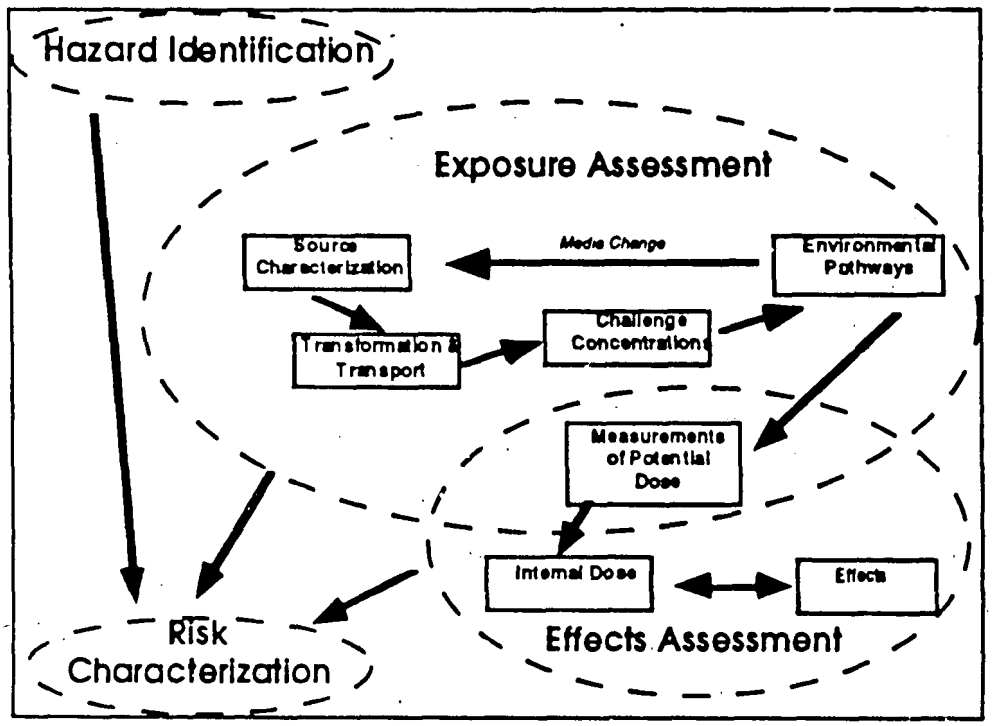

Figure 1: Simplified Exposure Assessment Paradigm. A substance is released to the environment, is transported, may be transformed chemically and physically, and can move through various pathways; e.g., water, air, soil and sediment. After a substance reaches an environmental pathway; i.e., media change, 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.

EXPOSURE PROTOCOL FOR ENDOCRINE DISRUPTORS

The U.S. Environmental Protection Agency (7) classifies dose as: potential dose (D_P); applied dose (D_A); internal dose (D_I); delivered dose (D_D); and biologically effective dose (D_{BE}). Figure 2 shows the path from 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_D are most often expressed by biomarkers, i.e., "indicators of changes or events in human biological systems" (8). 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" (9). Biomarkers can also apply to ecological exposure, although they are not often classified as measures of dose ("biotic and abiotic accumulation" in Figure 4). For example, Hunsaker et al (10) have suggested measuring cholinesterase levels and porphyrin accumulation to indicate the level of ecosystem exposure.

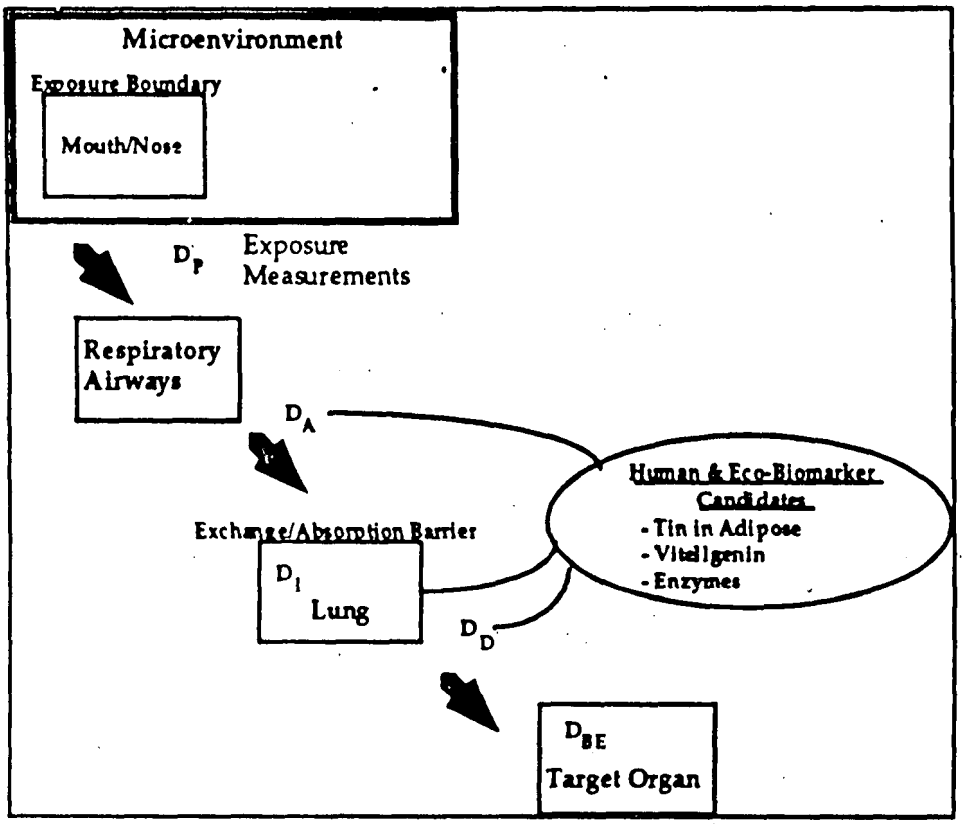

Figure 2: U.S. Environmental Protection Agency's Schematic of Dose and Exposure for Airborne Substances (Modified by McCurdy (11)). Biomarkers can be substances to which the organism is exposed or metabolites (e.g., enzymes) indicating exposure.

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 (12) expressions of LADD, total LADD may be calculated as the sum of all LADD values via all routes:

$$LADD_T = LADD_A + LADD_I + LADD_D$$

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 must be further, subdivided. For example, $LADD_T = LADD_g + LADD_p$

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

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)

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

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.

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. Although endocrine disruption risk assessment shares many elements with other types of toxicity, endocrine disruptor exposure is further 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 and may be expressed as:

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

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

Exposure models should incorporate physico-chemical 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 different idealized bimodal distributions for particles. The distributions can provide weight-of-evidence for whether the particles are anthropogenic or natural in origin. This research has recently experienced significant advances; e.g., Karickhoff and Long (14) 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). The models should run at appropriate spatial and temporal scales inherent in multimedia transport systems which have been identified as critical to exposure assessment (i.e., timing and duration of exposure).

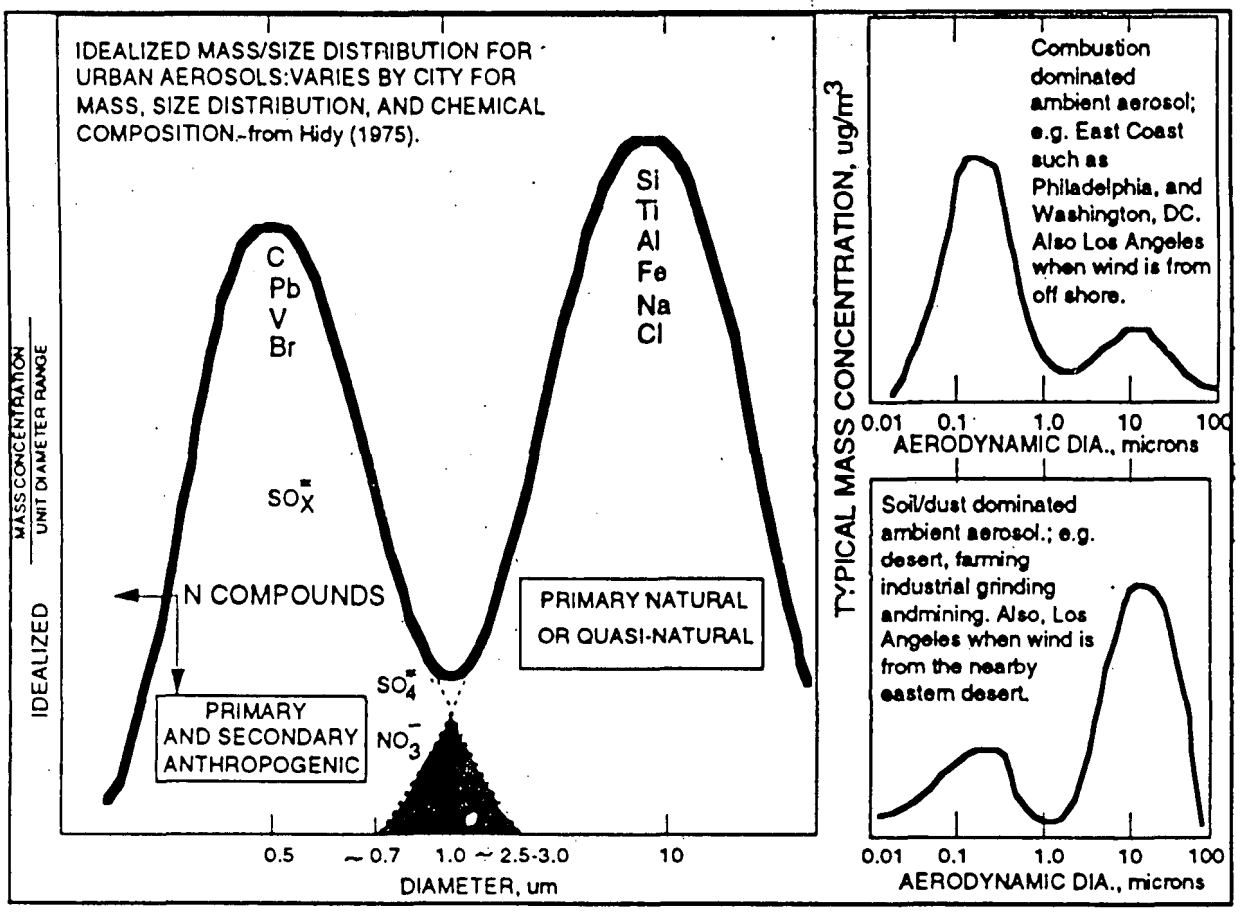

Figure 3: Particles often display a bimodal distribution by mass, originate from multiple sources, show dynamic growth and reactivity, and are carriers of other pollutants (Hidy (13)). The upper right mass distribution is typical for an area dominated by anthropogenic (combustion) sources, while the bottom distribution is typical for areas where particles are generated from noncombustion sources (e.g., re-entrained soil and mining activities).

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 (15). A major difference between human and ecological exposure paradigms is their level of biological organization; i.e., population exposure for one species (human) versus community (several species), association, and population exposure for ecological risk assessments (16). However, as indicated in Figure 4, a number of similarities exist between human and ecological exposure assessments. Both 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).

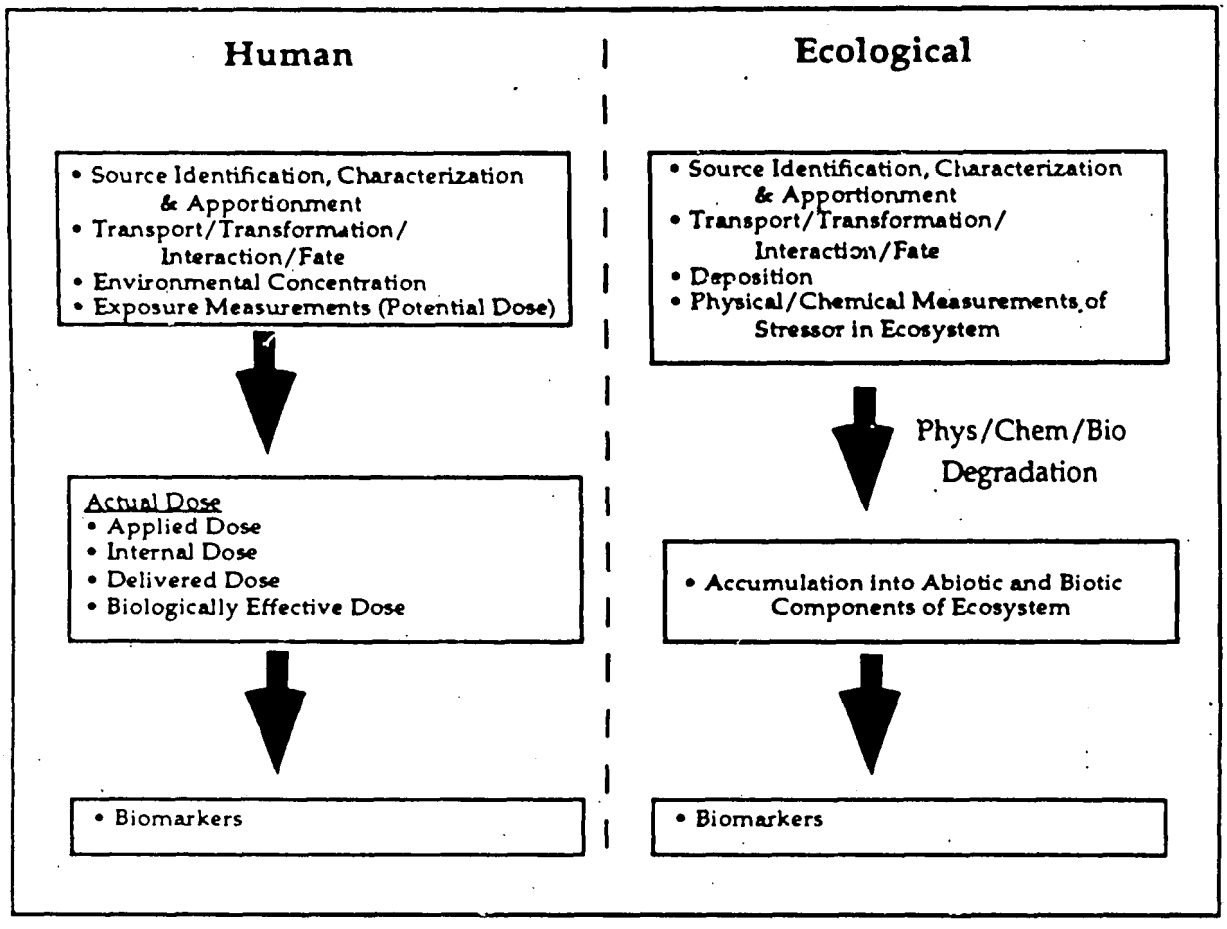

Figure 4: Exposure components of risk paradigms are similar for humans and ecosystems (16).

ENDOCRINE DISRUPTOR EXPOSURE RESEARCH AREAS

The National Academy of Sciences (8) has recommend approaches for assessing human exposure to airborne pollutants (Figure 5), illustrating 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 physico-chemical characterization of known or highly suspect endocrine disruptors. Estimating exposures 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 the major focus of this research.

Chemical substances 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 physico-chemical 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 et al (17, 18, 19), have developed models to simulate the movement and change of chemical substances in the environment, as a function of their physico-chemical 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.

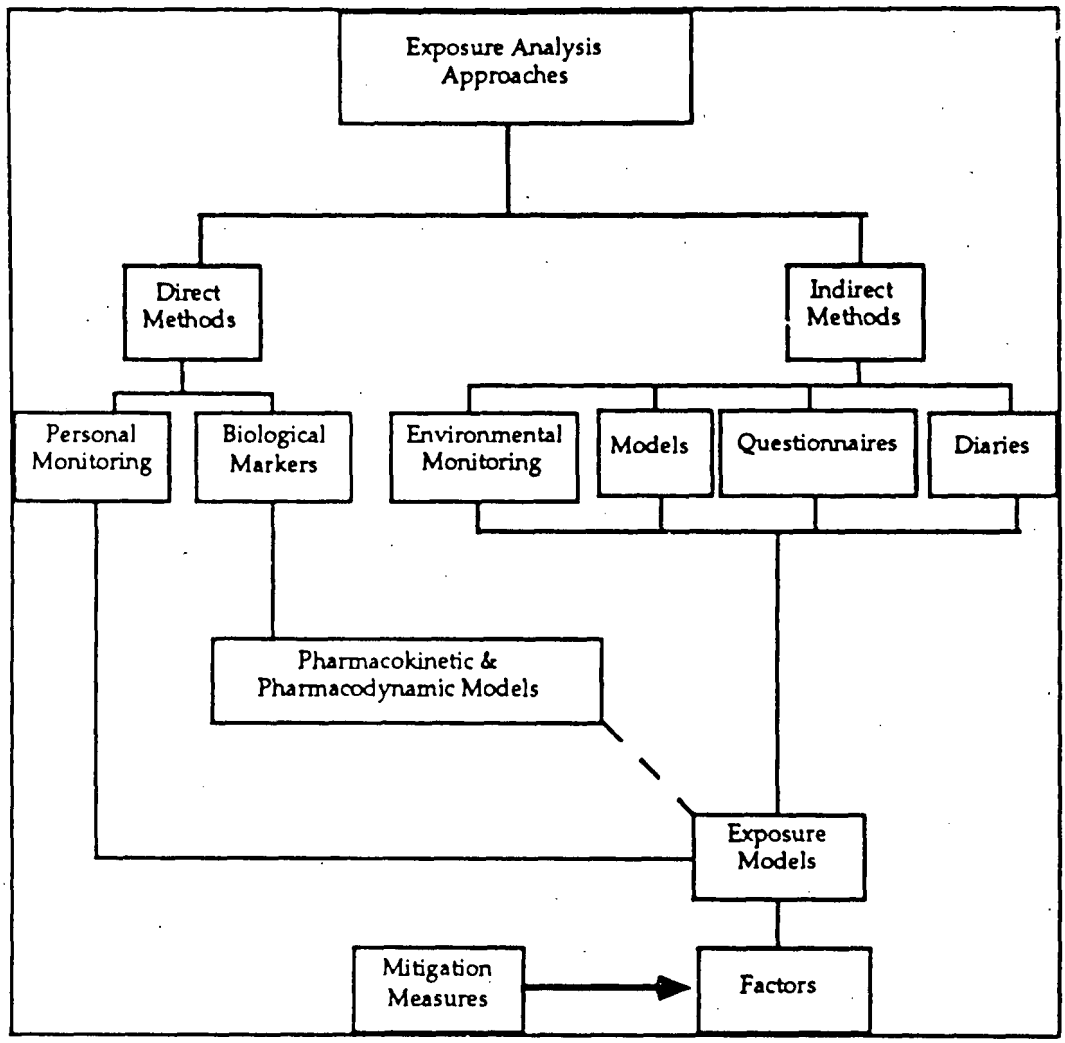

Figure 5: Possible approaches for analysis of air contaminant (8). The dashed line between PK and PD models and exposure models added by author to show that exposure models can be derived from direct measurement data, from routines from other models, and from combinations of measured and derived data.

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 the ionizable endocrine disruptors, under varying pH and ionic strength conditions.

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.

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 NBS, 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 the models. Improved toxicokinetic and structure activity models need to be linked with the physico-chemical 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. Effects and exposure biomarkers must be calibrated to adverse individual- and population-level effects. 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.

SCALE OF EXPOSURE

Endocrine disruptor research will take place at 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.

In the case of the small-scale assessment (residential, occupational, farms), 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.

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).

EXPOSURE HYPOTHESIS TESTING SITES

A potentially productive research approach would be to comprehensively investigate a small number of experimental sites/systems with problems that are known or strongly-suspected to be related to endocrine disruptors. This type of integrated study, conducted at multiple levels of biological organization, with both laboratory and field components, could yield significant insights into many of the issues mentioned above, including 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. Pilot study sites could be selected based upon strong weight-of-evidence that populations have been affected by exposure to endocrine disruptors. Such evidence can consist of ecological epidemiology, positive response or exposure screens, historical data suggesting a likelihood that endocrine disruptors are present in one or more environmental media, or where source or fate models suggest a "hot spot." An ecosystem approach should be adopted and multiple phylogenetic groups and trophic levels should be studied at a given site.

Such studies may also be used to test and validate 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 near each of its facilities, which may help fill this need. At a minimum, these "near-laboratory" sites will provide a means for developing and testing measurement protocols for a wide range of pollutants, including those suspected of disrupting endocrine function.

CONCLUSIONS

As researchers begin to relate environmental exposures to endocrine effects in humans and wildlife, they will need will need increasingly reliable data and models. The compartmental models show promise in identifying major knowledge gaps and uncertainties for chemical classes which may be associated with endocrine disruption. These models make use of physical and

chemical characteristics of substances and, as such, improved understanding of how substances behave in the various environmental compartments is crucial to exposure assessments. The modeling should be complemented by field data from pilot studies of areas where exposure and effects hypotheses can be tested, especially weight-of-evidence of suspect endocrine disruptors can be compared effects and biological responses in humans and wildlife.

REFERENCES

1. Colborn, T and Clement, C, editors. Chemically induced alterations in sexual and functional development: The wildlife/human connection. *Advances in Modern Environmental Toxicology*. Vol. XXI, Princeton Scientific Publishing, Princeton, NJ. 401pp. (1992).
2. Adams, NR. Organizational and activational effects of phytoestrogens on the reproductive tract of the ewe. *PSEBM* 208:87-91 (1995).
3. Stob, M. Naturally occurring food toxicants: Estrogens. IN: *Handbook of Naturally Occurring Chemicals*, M. Recheigl, editor, CRC Press, Boca Raton, Florida, pp 81-102 (1983).
4. Bern, H.A. The Fragile Fetus. IN: Chemically induced Alterations in Sexual and Functional Development: The Wildlife-Human Connection. Colborn, T., Clement, C. editors. vol. 21. Princeton Scientific Publishing, Princeton, N.J. (1992)
5. Kavlock R.J., Daston G.P., DeRosa C., Fenner-Crisp P., Gray L.E., Kaattari S., Lucier G., Luster M., Mac M.J., Maczka C., Miller R., Moore J., Rolland R., Scott G., Sheehan D.M., Sinks T., and Tilson H.A. (1996). Research Needs for the Risk Assessment of Health and Environmental Effects of Endocrine Disruptors: A Report of the US EPA Sponsored Workshop. *Environmental Health Perspectives* (in press).
6. Natural Research Council. *Risk Assessment in the Federal Government: Managing the Process*. Washington D.C. National Academy Press. 191 pp. (1983).
7. U.S. Environmental Protection Agency (1992). *Federal Register*. Guidelines for Exposure Assessment. Vol. 57. No. 104. FRL-4129-5.
   U.S. Environmental Protection Agency.
8. National Academy of Sciences, National Research Council (1991). *Human Exposure Assessment for Airbone Pollutants: Advances and Opportunities.* Washington, DC: National Academy Press.
9. Huggett, R.J., Kimerle, R.A., Mehrle, Jr., P.M., and Bergman, H.L. eds. 1992. *Biomarkers-Biochemical, Physiological, and Histological Markers of Anthropogenic Stress.* Boca Raton, LA: Lewis Publishers.
10. Hunsaker, C.T., MacCarthy, J.F., Shugart, L.R., and O'Neill, R.V. (1990). Indicators Relevant to Multiple Resource Categories. In *Ecological Indicators for the Environmental Monitoring and Assessment Program*. Hunsaker, C.T and Carpenter, D.E., editors , 2-1 - 2-18. EPA 600/3-90/060. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development.
11. McCurdy, T. (Draft in Process). Modeling the "Dose Profile" in Human Exposure Assessments: Ozone as an Example. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development.
12. Derelanko, M.J. (1995). Risk Assessment Calculations. IN: *CRC Handbook of Toxicology*. Derelanko, M.J. and Hollinger, M.A. editors. CRC Press, Boca Raton, 653-656.
13. Hidy, G.M. (1975). Summary of the California Aerosol Characterization Experiment. *J. Air Poll. Cont. Assoc.* 25: 1106-1114.
14. Karickhoff, S.W. and Long, J.M. (1996). Research Program: SPARC. National Exposure Research Laboratory Briefing.

15. Environmental Monitoring and Assessment Program (1993). *Environmental Monitoring and Assessment Program: Master Glossary*. EPA/620/R-93/013. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development.
16. Vallero, D.A. (1996). Exposure Assessment Methodologies for Humans and Ecosystems. IN: *NATO ASI Series, Partnership Sub-Series, 2. Environment: Urban Air Pollution*. Allegrini, I. and De Santis, F. editors. Springer-Verlag: Berlin Heidelberg, 8: 449-466.
17. Cohen, Y., Wangteng, T. and Chetty, S. (1990). A Course on Multimedia Environmental Transport, Exposure, and Risk Assessment. *ChE Curriculum*. Fall 1990: 212-219.
18. Cohen, Y. and Allen, D. (1992). An Integrated Approach to Process Waste Minimization Research. *J. Haz. Materials*. 29: 237-253.
19. Cohen, Y. and Clay, E.C. (1994). Multimedia Partioning of Particle-Bound Organics. *J. Haz. Materials*. 37: 507-526.

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