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Research Needs for the Risk Assessment of Health and Environmental Effects of Endocrine Disruptors: A Report of the U.S. EPA-sponsored Workshop

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The hypothesis has been put forward that humans and wildlife species have suffered adverse health effects after exposure to endocrine-disrupting chemicals. Reported adverse effects include declines in populations, increases in cancers, and reduced reproductive function. The U.S. Environmental Protection Agency sponsored a workshop in April 1995 to bring together interested parties in an effort to identify research gaps related to this hypothesis and to establish priorities for future research activities. Approximately 90 invited participants were organized into work groups developed around the principal reported health effects—carcinogenesis, reproductive toxicity, neurotoxicity, and immunotoxicity—as well as along the risk assessment paradigm—hazard identification, dose–response assessment, exposure assessment, and risk characterization. Attention focused on both ecological and human health effects. In general, the group felt that the hypothesis warranted a concerted research effort to evaluate its validity and that research should focus primarily on effects on development of reproductive capability, on improved exposure assessment, and on the effects of mixtures. This report summarizes the discussions of the work groups and details the recommendations for additional research. — Environ Health Perspect 104(Suppl 4):715–740 (1996)

Key words: endocrine disruptors, hormones, risk assessment, carcinogenesis, reproductive toxicity, developmental toxicity, immunotoxicity, neurotoxicity, exposure assessment, research needs

Introduction

Evidence has been accumulating which indicates that humans and domestic and wildlife species have suffered adverse health consequences from exposure to environmental chemicals that interact with the endocrine system (e.g., *1–3*). To date, these health problems have been identified primarily in domestic or wildlife species with relatively high exposures to organochlorine compounds, including 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites, polychlorinated biphenyls (PCBs) and dioxins, or to naturally occurring plant estrogens. It is not known if similar effects are occurring in the general human population, but again there is evidence of adverse effects in populations with relatively high exposures. Several reports (*4*) of declines in the quality and decreases in the quantity of sperm production in humans over the last four decades and reported increases in incidences of certain cancers (breast, prostate, testicular) that may have an endocrine-related basis have led to speculation about environmental etiologies. However, considerable scientific uncertainty remains regarding the causes of these reported effects. Nevertheless, it is known that the normal functions of all organ systems are regulated by endocrine factors, and small disturbances in endocrine function, especially during certain stages of the life cycle such as development, pregnancy, and lactation, can lead to profound and lasting effects. The critical issue is whether sufficiently high levels of endocrine-disrupting chemicals exist in the ambient environment to exert adverse health effects on the general population.

Current methodologies for assessing human and wildlife health effects (e.g., the generation of data in accordance with testing guidelines developed by the U.S. Environmental Protection Agency [U.S. EPA]) are generally targeted at detecting effects rather than mechanisms, and may not adequately evaluate effects on the endocrine system. This is particularly true for exposures that occur during critical developmental periods when the endocrine system plays a key role in regulating essential physiological and morphological processes. Given the potential scope of the problem, the possibility of serious adverse effects on the health of human and wildlife populations, and the broad occurrence and persistence of some endocrine-disrupting agents in the environment, it is important to focus the available resources for research on the most critical gaps in our knowledge

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Abbreviations used: CNS, central nervous system; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-,bis(*p*-chlorophenyl)ethane; DES, diethylstilbestrol; DHEA, dehydroepiandrosterone; EDC, endocrine-disrupting chemical; EROD, ethoxyresorufin-*O*-deethylase; LH, luteinizing hormone; MUNG, *N*-methyl-*N*-nitroso-*N'*-nitroguanidine; NTP, National Toxicology Program; NOAEL, no observed adverse effect level; PAHs, polyaromatic hydrocarbons; PBBs, polybrominated biphenyls; PCBs, polychlorinated biphenyls; PCDFs, polychlorinated dibenzofurans; QSAR, quantitative structure–activity relationship; SAR, structure–activity relationship; SEER, surveillance epidemiology and end results; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEF, toxic equivalency factor; TEQ, toxic equivalent approach; TIE, toxicity identification evaluation.

base so that more informed regulatory and public health decisions can be made in the future. The broad nature of the problem necessitates a coordinated effort on both the national and the international levels. The National Science and Technology Council, which advises the president and his Cabinet on directions for federal research and development efforts, has established a milestone for 1995 to 1998 to produce a national research strategy on endocrine-disrupting chemicals. Therefore, in response to the growing public health concerns related to chemicals in the environment that have the potential to act as endocrine disruptors, the Office of Research and Development of the U.S. EPA held a workshop on April 10–13, 1995, in Raleigh, North Carolina, to begin developing a national research strategy related to endocrine-disrupting chemicals. An organizing committee was formed that consisted of representatives from various organizations, including the U.S. EPA, Department of Health and Human Services, Department of the Interior, Department of Commerce, and Department of Agriculture; industrial groups such as the Chemical Manufacturers Association and the American Industrial Health Council; independent organizations such as the Institute for Evaluating Health Risks; and public interest groups such as the World Wildlife Fund and the Environmental Defense Fund.

The premise of the workshop was as follows: because environmental endocrine disruptors have caused a variety of adverse biological effects in wildlife species, domestic animals, and humans, we need to identify specific research that would assist the federal government in making informed decisions. An environmental endocrine disruptor was broadly 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." This definition reflects a growing awareness that the issue of endocrine disruptors in the environment extends considerably beyond that of exogenous estrogens and includes antiandrogens and agents that act on other components of the endocrine system such as the thyroid and pituitary glands. Approximately 90 invited participants and members of the organizing committee (Table 1) were asked to discuss research needs related to the principal adverse health effects reported for endocrine disruptors (carcinogenesis, reproductive [including developmental] toxicity, immunotoxicity, and neurotoxicity) as well as research to improve specific components of the risk assessment paradigm (hazard identification, dose–response assessment, exposure assessment, and risk characterization). Attention focused on both ecological and human health effects. A series of questions was posed to each group to help guide the discussions. More than 200 observers from academia, industry, governmental organizations, public interest groups, and the press also attended the workshop. This report summarizes the major findings of each discussion group.

Dr. Lynn Goldman, Assistant Administrator, Office of Prevention, Pesticides and Toxic Substances (OPPTS), U.S. EPA, opened the workshop. She reviewed the impact of the National Performance Review on environmental protection, the process of updating the OPPTS testing guidelines, and some regulatory activities related to endocrine disruptors (e.g., the special review of triazines, an evaluation of endosulfan, and the status of the alkylphenol ethoxylate consent order). Her presentation emphasized the need to address the major scientific and policy questions as the foundation for mitigating the potential impact of endocrine disruptors. Dr. Howard Bern (University of California at Berkeley) gave the keynote address. This historical perspective was based largely on the human diethylstilbestrol (DES) syndrome and on the neonatal mouse model used in its experimental analysis. He emphasized long-term permanent effects in the adult as a result of exposure to agents during development, which can occur without apparent birth defects in the neonate. Dr. Bern also emphasized the concept of critical periods for epigenetic effects on different targets and indicated the wide range of organs and physiological systems that may be affected—reproductive, endocrine, immune, neural, behavioral, metabolic, skeletal, etc. The particular sensitivity of systems to endocrine-disrupting agents during development implies that embryonic, fetal, and neonatal tissues may "see" estrogens, estrogen-mimics, and other endocrine disruptors in a different way (perhaps even by different mechanisms) than adult tissues. As a final prelude to the discussions, representatives from Germany (Dr. Andreas Gies, Umwelt Bundes Amt), the United Kingdom (Dr. Linda Smith, Department of the Environment), and Denmark (Dr. Jorma Toppari, University of Turku, Turku, Finland, on behalf of the Danish Ministry of Environment and Energy and the Danish Environmental Protection Agency) presented summaries of research needs identified by their respective governments in recent workshops (*4–6*).

General Comments from the Work Groups

Each work group consisted of individuals with various backgrounds including field ecology, epidemiology, basic sciences, animal and human toxicology, exposure assessment, and risk assessment. This mix of experts was perceived as a great advantage in enhancing the groups' ability to look at the overall problem, stimulating establishment of common priorities, and identifying new solutions to existing methodologic issues. An interdisciplinary approach should be maintained in any follow-up action to this workshop. Consistent with the interdisciplinary approach, it is important that methods and results found in one research arena be applied to other research arenas. Basic research (e.g., mechanistic discoveries in cancer etiology) should be applied to observational research (e.g., use of DNA polymorphisms in epidemiological or field ecology studies) and vice versa (e.g., identification of the mechanisms by which DES increases clear-cell carcinoma of the vagina in women). Sound scientific information must be the basis of good decision making. We should be careful not to overinterpret study results when the basic scientific techniques have not been standardized or validated. The use of biologic measurements requires an understanding of good laboratory practices (QA/QC), reproducibility, accuracy, statistical power, and replicability. In addition, interpretation that some measurements are predictive of adverse biological effects requires validation studies. Finally, the underlying issue of dose response must always be considered when results observed over a limited dose range are evaluated .

Many of the gaps in our understanding of dose–response relationships for endocrine disruptors are the same as those that have led to much uncertainty and associated controversy arising from using default assumptions made by regulatory agencies in the risk assessment process. The validity of assumptions used in risk assessments is frequently challenged when high-dose data in experimental systems are used to estimate effects at much lower doses in humans. Our growing analytical ability to detect chemicals at continually lower concentrations, coupled

Table 1. Work group participants and assignments.

Participant[a]	Affiliation[b]	Group[c]	Participant[a]	Affiliation[b]	Group[c]
Mel Anderson	ICF Kaiser	C,DR	Garet Lahivs	University of Maryland–Baltimore	I,DR
Gerald Ankley[a]	U.S. EPA	R,HD	Coral A. Lamartiniere	University of Alabama–Birmingham	C,EX
Christopher J. Bayne	Oregon State University	I,DR	John F. Leatherland	University of Guelph	N,EX
David Bellinger	Boston's Children's Hospital	N,DR	Jonathan J. Li	University of Kansas Medical Center	C,EX
Howard A. Bern	University of California–Berkeley	R,HD	George Lucier[a]	NIEHS	C,DR†
Linda Birnbaum	U.S. EPA	C,EX	Mike Luster	NIEHS	I‡,DR
Aaron Blair	NCI	C,HD	Michael J. Mac[a]	National Biological Service	R,EX†
Joanna Burger	Rutgers University	N,HD	Neil J. MacLusky	The Toronto Hospital	N,RC
Cynthia Carey	University of Colorado	I,EX	Carol Maczka	National Research Council	R,HD‡
Janice E. Chambers	Mississipi State University	N,HD	Peter Mathiessen	MAFF, United Kingdom	R,EX
Robert E. Chapin	NIEHS	R,RC	Lynne F. McGrath	Hoechst–Celanese	I,DR
James R. Clark[a]	Exxon Biomedical Sciences	R,RC	John McLachlan	Tulane University	C,RC
Theodora Colborn[a]	World Wildlife Fund	I,EX	Sue McMaster[a]	U.S. EPA	N,RC
Rory Conolly	Chemical Industry Institute of Toxicology	C,DR	Mark S. Meyers	Northwest Fisheries Science Center	C,RC
Jon Cook	Dupont-Haskell Laboratory	C,HD	Diane Miller	U.S. EPA	N,RC
Ralph Cooper	U.S. EPA	N,HD	Ron R. Miller[a]	DOW Chemical Co.	C‡,DR
John Couch	U.S. EPA	C,HD	John A. Moore[a]	IEHR	I,RC†
David Crews	University of Texas at Austin	N,RC	Larry L. Needham	CDC	C,EX
Sally Darney	U.S. EPA	R,RC	Reynaldo Patino	National Biological Service	R,EX
George Daston[a]	The Procter and Gamble Co.	R†,DR	Richard E. Peterson	University of Wisonsin–Madison	R,RC
William P. Davis	U.S. EPA	R,EX	Warren P. Porter	University of Wisconsin–Madison	N,RC
Chris DeRosa[a]	ATSDR	C,EX‡	Christopher J. Portier	NIEHS	I,DR
Penelope Fenner-Crisp	U.S. EPA	C,RC‡	Walter Rogan	NIEHS	N,RC
Warren Foster	Environmental Health Canada	R,RC	Rosalind M. Rolland[a]	World Wildlife Fund	R‡,HD
Michel Fournier	Université du Québec á Montréal	I,DR	Louise M. Ryan	Harvard University	R,DR
Glen Fox	CWS, Environment Canada	I,EX	Geoffrey I. Scott[a]	National Marine Fisheries Service	N‡,DR
D. Michael Fry	University of California–Davis	R,HD	R. Woodrow Setzer	U.S. EPA	R,DR
Michael A. Gallo	Robert Wood Johnson Medical School	RC	Daniel M. Sheehan[a]	NCTR/FDA	R,DR‡
David Gaylor	NCTR	N,RC	Barbara B Sherwin	McGill University	N,RC
Ellen Goldey	U.S.EPA	N,HD	Ellen K. Silbergeld[a]	University of Maryland–Baltimore	N,EX
Tom Goldsworthy	Chemical Industry Institute of Toxicology	C,DR	Thomas Sinks[a]	CDC	C†,EX
Jay Gooch	The Procter and Gamble Co.	R,HD	Ralph Smialowicz	U.S. EPA	I,HD
L. Earl Gray Jr[a]	U.S. EPA	R,HD†	George Stancel	University of Texas Medical School	R,DR
Louis J. Guillette	University of Florida–Gainesville	R,HD	John J. Stegemen	Woods Hole Oceanographic Institute	C,EX
Maureen C. Hatch	Mt. Sinai School of Medicine	R,EX	Peter Thomas	University of Texas at Austin	R,DR
Jerry D. Hendricks	Oregon State University	C,HD	Donald Tillitt	National Biological Service	R,EX
Andrew G. Hendrickx	University of California–Davis	R,RC	Hugh Tilson	U.S. EPA	N†,HD
Diane Henshel	Indiana University	N,DR	Jorma Toppari	University of Turku, Finland	R,ID
David E. Hinton	University of California–Davis	C,EX	Kamala Tripathi[a]	USDA	I,EX
Mike Holsapple	DOW Chemical Co.	I,RC	Daniel A. Vallero	U.S. EPA	N,EX
Claude L. Hughes Jr	Wake Forest University	R,DR	Frederick S. Vom Saal	University of Missouri–Columbia	R,DR
Lyndal Johnson	Northwest Fisheries Science Center	R,RC	Chris Waller	U.S. EPA	C,HD
Rod Johnson	U.S. EPA	C,RC	Patricia Whitten	Emory University	R,DR
Steven L. Kaattari	ViMS, College of William and Mary	I†,RC	Elizabeth Wilson	University of North Carolina–CH	R,HD
William Kelce	U.S. EPA	R,HD	Judith T. Zelikoff	New York University Medical Center	I,EX
Carole Kimmel	U.S. EPA	R,RC			

[a]Organizing committee members (Dick Hill, U.S. EPA, and Jim Reisa, NRC, were unable to attend). [b]ATSDR, Agency for Toxic Substances and Drug Reserch; CDC, Centers for Disease Control; CWS, Canadian Wildlife Service; FDA, Food and Drug Administration; ICF Kaiser; MAFF, Ministry of Agriculture, Fisheries and Food (United Kingdom); NCI, National Cancer Institute; NCTR, National Center for Toxicological Research; NIEHS, National Institute of Environmental Health Sciences; U.S. EPA, U.S. Environmental Protection Agency. [c]C, carcinogenic effects, R, reproductive effects; N, neurological effects; immunological effects; H D, hazard detection methods; DR, dose–response methods; EX, exposure methods; RC, risk characterization methods; †, chairperson; ‡ rapporteur.

with the availability of molecular approaches to detect chemical interactions with biological systems, is creating opportunities and more tractable approaches to improve low-dose risk estimates. Yet considerable work remains to be done at the laboratory and science policy levels before there is widespread acceptance of mechanistic data in quantitative risk assessments. We need to improve the use of existing data and to have better data and predictive models to strengthen the scientific foundation for estimating dose–response relationships for endocrine disruptors.

Several considerations are essential to the examination of the effects of potential endocrine disruptors. First is the consideration of the different sensitivities at different ages. In general, the developing organism is especially sensitive, for example, DES induction of adenocarcinoma of the vagina in females exposed before the end of the first trimester of their mother's pregnancy. The second consideration is a direct consequence of the first. Because the work group recognized the importance of the developmental stage at exposure, exposure assessment as defined by the National Research Council (7) was modified (change noted in italics) to read: "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 chemi-

cals." The third consideration is that adverse effects can arise from either primary or secondary disturbances of endocrine function. Thus, an indirect-acting endocrine disruptor affects a systemic target organ first; these effects in turn may influence the endocrine system to cause secondary neurotoxicity, reproductive toxicity, and/or immunotoxicity. Conversely, a direct-acting endocrine disruptor affects the endocrine system first, which in turn results in toxicity in other organ systems. In some aspects it is very difficult, if not impossible, to separate the endocrine system from the systemic target organs, so this distinction should be viewed in an abstract manner. Obviously, some chemicals can adversely affect the structure and/or function of the systemic organs without any endocrine system involvement. Such chemicals may be considered direct-acting target organ toxicants. These relationships are portrayed in Figure 1. Finally, the development of a research agenda for endocrine disruptors should be a national or international effort by many agencies, not just the U.S. EPA, so that the limited resources can be used in the most efficient manner.

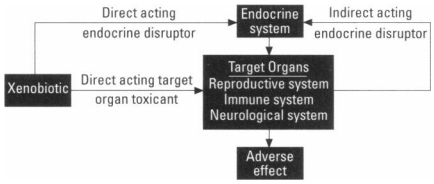

Figure 1. Abstract representation of the interplay between the endocrine system and the reproductive, neurological and immunological systems to illustrate the complexity of determining the mode of action for chemicals that cause adverse effects through involvement of the endocrine system.

Biological Effects Issues

Carcinogenic Effects

What do we know about the carcinogenic effects of endocrine-disrupting agents in humans and wildlife? What are the major classes of chemicals thought to be responsible for these effects? What are the uncertainties associated with the reported effects?

Numerous field studies of teleost fishes in localized highly contaminated areas (i.e., "hot spots") have shown high prevalences of liver tumors (*8–10*). The predominant risk factor that has been associated with these liver tumors is exposure to polyaromatic hydrocarbons (PAHs) and to a lesser degree, PCBs and DDT. Certain species such as carp and fathead minnows are more resistant, while trout are more sensitive. There has been no indication that the liver tumors in fish involve an endocrine modulation mechanism. Other than for localized areas of high contamination, field studies have shown no increasing trends for tumors of any type in fish. The group noted that two tumor registries for wildlife species exist in the United States (the Smithsonian Registry of Tumors in Lower Animals under the direction of Dr. John Harshbarger, and the Armed Forces Institute of Pathology's Registry of Comparative Pathology under the direction of Dr. Linda Johnson). A variety of dose-related tumors can be produced in fish given carcinogens under experimental laboratory conditions (*11*). Again, there is no specific evidence that the development of these tumors involves a hormonal disruption mechanism. Estradiol and certain hormone precursors (e.g., dehydroepiandrosterone [DHEA]) act as promotors after treatment of fishes with carcinogenic substances such as aflatoxin and *N*-methyl-*N*-nitroso-*N'*-nitroguanidine (MNNG). Toxicopathic liver lesions have been associated with contaminant exposure in some marine fish (*10*).

There is a paucity of carcinogenicity data for other forms of wildlife. One study of beluga whales in the St. Lawrence seaway found that approximately 50% of dead whales examined had neoplasms, of which about 25% were malignant (*12,13*).

The hypothesis that endocrine disruption can cause cancer in humans is based on the causal association between DES exposure of pregnant women and clear-cell adenocarcinoma of the vagina and cervix in their female offspring, hormone-related risk factors for breast and uterine cancer, and limited evidence of an association between body burden levels of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) or PCBs and breast cancer risk. Young women who developed cancer of the vagina were more likely to have had mothers who used DES during pregnancy to avoid miscarriage than mothers who did not use the drug (*14*). This finding has led to a number of important conclusions. First, maternal exposures during gestation can lead to cancer in offspring, and second, it demonstrates that a synthetic estrogen can cause cancer. Some of the male offspring of women who took DES display pseudohermaphroditism (*15*) and genital malformations, including epididymal cysts, testicular abnormalities such as small testes and microphallus, and reduced semen quality (*16–18*). Follow-up surveys of DES-exposed male offspring, however, have not shown impairment in fertility or sexual function (*19,20*), nor is there evidence of increased risk of testicular cancer (*19*).

The most common cancer among women in the United States is breast cancer. A number of epidemiological studies have examined the risk factors for breast cancer. Identified risk factors include several that relate to hormonal activity: decreased parity, age at first delivery, age at menarche, age, race, and unopposed estrogen therapy. In addition, breast tumors can be characterized as to their degree of estrogen-receptor positivity resulting in relevant prognostic information. The evidence supports a causal relationship between female breast cancer and hormonal activity.

A number of organochlorine pesticides or pesticidal metabolites are found in breast milk and human adipose tissue (*21,22*). Several recent cross-sectional studies suggest a possible relationship between levels of some organohalide residues in human tissues and breast cancer risk, although the observations are not entirely consistent across studies, and no clear relationship has been established (*23–30*). In general, these studies suggested that levels of *p,p'*-DDE and total PCBs were higher in fat or serum of women who had breast cancer than in comparison groups. The meaning of these findings is unclear, in part, because *p,p'*-DDE and the few PCB congeners that have been tested have little or no discernible estrogenic activity, while the short-lived forms of DDT, *o,p'*-DDT and *o,p'*-DDE, have only very weak estrogenic properties. Further, a recent case–control study with historical data from serum DDE and PCBs conflicts with the earlier findings (*29*). This study showed no overall effect of serum residue levels on breast cancer risk, although subcategorical analysis did suggest a possible increase in risk among black women with higher levels of serum *p,p'*-DDE. The women in these studies, except those in the study by Henderson et al. (*30*), were not exposed to high levels of PCBs or DDE and the actual differences in levels measured between cases and controls were not large. Studies of women occupationally exposed to high levels of PCBs have not demonstrated an excess risk of breast cancer mortality (*31,32*). The results of these studies therefore are equivocal and further research is needed (including examination of effects in subsequent generations from parental exposures).

Relatively good information exists on cancer occurrence (incidence) and mortality in the United States over the last several decades. The best incidence data come from the Surveillance Epidemiology and End Results (SEER) cancer registries that are supported by the National Cancer Institute (*33*). Cancer trend data from 1973 to 1991 show that age, race, and sex-adjusted total cancer incidence increased by 31% in males and 14% in females. Incidence rates between 1973 and 1991 for several hormone-sensitive tissues have increased (female breast 24%, ovarian 4%, testicular 41%, prostate 126%) as have several other cancer sites: melanoma (116% in males, 73% in females); non-Hodgkins lymphoma (84% in males, 57% in females); and liver (55% in males, 27% in females). However, increases in female breast and male prostate cancers account for the majority of total cancer increases experienced by women (52%) and men (70%) during this time period. The increases in testicular and ovarian cancer represent only 1% of the total increase in cancer incidence. SEER data indicate that incidences of uterine cancer and male breast cancer have remained constant or declined slightly.

Cancer screening is available for breast and prostate cancer. Recent advances in the screening process account for some of the reported increases in cancer incidence. White et al. (*34*) reported that although the increase in breast cancer incidence for women 25 to 44 years of age can be explained by screening, screening does not entirely explain the increase for younger or older women. Feuer and Wun (*35*) suggest that screening may also account for all of the observed increase in older women. It has also been suggested that increased detection of prostatic cancer is due to increased screening for prostate-specific antigen (PSA) (*36*).

Examination of the U.S. EPA database on pesticide registration for organochlorines showed no correlation of the spectrum of tumor types observed in laboratory animals with the assertion that the organochlorines are related to human breast cancer. Organochlorines frequently increased the incidence of liver tumors in rats, but did not increase the incidence of mammary tumors (P. Fenner-Crisp, personal communication to work group). One subclass of herbicides, the chloro-*S*-triazines, produces an earlier onset of mammary tumors in Sprague-Dawley rats (*37,38*), but there are no epidemiological studies that suggest a relationship between exposure to triazine herbicide and human breast cancer. An examination of the National Toxicology Program (NTP) database involving approximately 450 animal studies showed increased incidences of mammary tumors in approximately 10% of the studies. However, based on evaluation of chemical structures and other available information, this subset of test substances is not likely to be estrogenic (*39*). This analysis only considered the possibility that the chemicals were direct estrogen agonists. It is obvious that other possible mechanisms exist for induction of endocrine-mediated tumors.

It was noted that good animal or cellular models do not yet exist for study of some endocrine-mediated tumors (e.g., testicular), which are reported to be on the increase in the human population, and that such models would be useful in testing cause-and-effect relationships.

What are the research needs related to the detection of carcinogenic effects of endocrine disruptors?

Most ecological field studies of cancer incidence have been devoted to examining fish in polluted waters. Some efforts have been made to establish comparison populations by examining nonpolluted waters. One study collected data that could be used for background information (*11*). There are no ongoing national surveillance programs for tracking cancer incidence and mortality in wildlife, but the Smithsonian Institution does maintain a tumor registry for fish and wildlife, and some marine mammal populations are being tracked for tumors (*12,13*). When populations of wildlife are evaluated, it is important to establish estimates of the expected occurrence of cancer. It is also important to identify special populations at high risk for cancer on the basis of high exposure or increased susceptibility. Background data could then be used for comparisons. Fish have proven to be the easiest animals to study. The occurrence of cancers in other animals should be examined as well.

In environmental settings, certain sentinel species may be useful. Sentinel species would include wildlife, domestic animals, or laboratory animals. The goal would be to identify species that are susceptible to developing cancer, easily monitored for cancer, and likely to reflect exposure in a single ecosystem.

Last, the evaluation of exposures should be expanded beyond polycyclic aromatic hydrocarbons and look at additional types of endocrine disruptors.

The study of the relationship between endocrine disruption and female breast cancer in humans is an obvious priority, not only because of the high incidence of this disease, but also because no primary prevention is available. Hopefully, the relationship between organochlorine exposure and breast cancer risk will be resolved soon, given the large number of ongoing epidemiological studies. Hence, focus should be placed on additional issues such as research on other possible environmental agents, including persistent and nonpersistent (e.g., nonorganochlorine pesticides and phthalates) environmental chemicals that may affect the endocrine system.

We should evaluate the relationship between exposure to endocrine disruptors and other cancer sites, particularly prostate, testicular, ovarian, endometrial, and thyroid. It is also important to evaluate cancers of the liver, brain, and lung, as these cancers are frequently associated with exposure to environmental hazards, although there is no evidence that exposure to EDCs is among the known primary risk factors for these tumors.

Epidemiological data on exposed human populations have proven useful for identification of human carcinogens. Occupational cohorts and cohorts of persons with exceptionally high environmental exposures [e.g., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Seveso, Italy (*40*); polychlorinated biphenyls (PBBs) in Michigan (*41*); and PCBs and polychlorinated dibenzofurans (PCDFs) in Japan (*42*) and Taiwan (*43*)] may be important for establishing a dose–response relationship. The highest priority is to identify, register, and follow populations with documented and quantitatively verified exposures.

There was discussion regarding the need to include *in utero* exposures in standard lifetime cancer bioassays to ensure protection of the developing offspring. The available evidence (*44–47*) does not clearly demonstrate a significant increase in sensitivity or induction of tumors compared to postnatal-only exposures. However, others have suggested that these retrospective comparisons are not adequate to determine the need for such exposures with EDCs, since that mechanism of action is underrepresented in the historical database.

Domestic animals and pets may be useful to identify carcinogenic hazards.

What are the highest priority research needs for carcinogenic effects?

The following high priority research needs were identified:

a) A systematic comparison of endogenous versus exogenous substances in terms of biologic activity, metabolism, structure–activity relationships, etc., with an initial focus on estrogenicity and rapid expansion to other steroid hormones. The exogenous estrogenic substances should include comparisons of phytoestrogens as well as other types of xenoestrogens in relation to endogenous estradiol.

b) Basic research to systematically and thoroughly evaluate species-, cellular-, and age-dependent responses, including consideration of mixtures of agonists, partial agonists, and antagonists, at environmentally relevant ratios and doses.

c) Careful evaluation of toxicity and mechanistic end points across species, including the determination of dose–response relationships in relation to human risk.

d) Surveillance data on the occurrence of tumors in wildlife species.

e) Identification and follow-up health studies of heavily exposed wildlife and human populations.

f) Validation and application of biomarkers that might be useful in identifying exposures as well as adverse outcomes based upon mechanistic considerations.

Other additional research recommendations included thorough consideration of the importance of critical timing (i.e., windows) of exposure before and after birth for carcinogenicity end points; determination of the role of metabolism (e.g., estradiol) in relation to certain toxicities such as breast cancer; identification of susceptibility factors such as polymorphisms that might result in a predisposition to certain end points, and assessment of structure–activity relationships for hormonal activity.

Reproductive Effects

What do we know about the reproductive and developmental effects of endocrine-disrupting agents in humans and wildlife? What are the major classes of chemicals thought responsible for these effects? What are the uncertainties associated with the reported effects?

Field and laboratory studies of wildlife populations and individuals have revealed effects in offspring that appear to be the result of endocrine disruption. Examples include reproductive problems in wood ducks from Bayou Meto, Arkansas (*48*); wasting and embryonic deformities in Great Lakes fish-eating birds (*49–56*); feminization and demasculinization of gulls (*57–60*); developmental effects in Great Lakes snapping turtles (*61*); embryonic mortality and developmental dysfunction in lake trout and other salminiods in the Great Lakes (*62–64*); abnormalities of sexual development in Lake Apopka alligators (*65,66*); reproductive failure in mink from the Great Lakes area (*67*); and reproductive impairment in the Florida Panther (*68*). In each case, detectable concentrations of chemicals with known endocrine-disrupting effects have been reported in the animals or in their environment, but an etiological link has been established for only a few of these observations. In ecological studies, these effects were not recognized until the populations began to decline. However, the observation that a population is stable is not an assurance that endocrine-disrupting chemicals are not affecting reproduction, development, and/or growth of individuals.

In humans, there is evidence of adverse reproductive outcomes in the DES sons and daughters (see "Carcinogenic Effects") and in male offspring from the Yu-Cheng poisoning incident (*69*). In adults, reproductive dysfunction has been observed in males exposed to kepone (*70*), and the duration of lactation has been reported to decrease as the concentration of DDE in the milk increases (*71,72*). Egeland et al. (*73*) reported decreased serum testosterone and increased luteinizing hormone (LH) levels in workers exposed to dioxin. There also have been reports of declining sperm quality in males over the last several decades (*74,75*), but the etiology is far from certain. The most convincing evidence for a general decline in male reproductive health in humans is the increase in testicular cancers noted over the recent past in several Western countries (*5*). Again, the contribution of environmental contaminants to this increased rate is unknown.

Further information on reproductive effects of endocrine-disrupting chemicals can be found in Colborn and Clement (*1*), Adams (*2*), Medical Research Council (*4*), Danish Environmental Protection Agency (*5*), Umweltbudesamt (*6*), McArthur et al. (*76*), Hoffman (*77*), Kihlstrom et al. (*78*), Colborn et al. (*79*), Jansen et al. (*80*), Jobling and Sumpter (*81*), Kelce et al. (*82*), Patnode and Curtis (*83*), Bergeron et al. (*84*), Guillette (*85*), Baldwin et al. (*86*), Dodson and Hanazato (*87*), Rolland et al. (*88*), and Kelce et al. (*89*). There are multiple targets for these chemicals, and effects are organ- and life stage-specific. Still other reports contain evidence of hormonal activity due to environmental chemicals without a direct link to reproductive effects (*90,91*).

Translating subtle functional deficits within individuals into population-level effects is the real challenge and will require better field observations and laboratory studies to more precisely simulate field exposures. The generalizations that can be made are grouped into the following categories:

a) Sensitive stages: Developmental stages are often the most sensitive to exposure. (Development in this instance is defined as broadly as possible to include embryonic, fetal, larval, and juvenile stages.) There are specific critical periods of sensitivity to endocrine disruption. These may be quite short and there may be more than one. Critical periods vary for different organs and species. The unique changes in physiology during development may increase sensitivity to endocrine-disrupting agents. Also, sensitivity may be sexually dimorphic or distinct in expression if not sensitivity.

Effects on development can be reversible because of effects on maturation or irreversible because of effects on differentiation. Often, irreversible effects on differentiation have a long latency for expression.

Adult males and females are also affected by endocrine disruptors, and there may be physiologic states in the adult (e.g., early pregnancy) that enhance susceptibility. Much of our knowledge of the action of endocrine disruptors in adult humans is derived from the use of various steroids as pharmacologic agents.

b) Types of effects: Endocrine disruptor effects vary by species and life stage at which exposure occurs. Endocrine disruption can result in morphologic abnormalities of the gonads, reproductive tract, brain, and other organs; functional and behavioral abnormalities; and certain malignancies (particularly of the reproductive system or related structures [e.g., breast]). Functional abnormalities include decreased semen quality, reduced numbers of sperm, infertility, disrupted estrous or menstrual cycling, and premature menopause; behavioral abnormalities include altered sexual behavior and decreased libido. Functional and behavioral abnormalities may occur after developmental or adult exposure, although the expression is likely to be different for the various life stages.

Circulating hormone concentrations may be altered by effects on hormone metabolism or steroidogenesis. There may also be effects on cellular function in the endocrine system or in systems that respond to the endocrine system mediated by toxicant interactions on different receptor classes.

At the more fundamental levels of biological organization, i.e., at the biochemical and cellular level, there tends to be a great deal of similarity among vertebrate classes due to the extreme phylogenetic conservation of hormones and hormone receptor binding characteristics. However, at higher levels of biological organization there may be divergence, as hormone systems may have different developmental functions in different phylogenetic groups. As an example, in the absence of androgens, the reproductive tract of mammals develops into a female phenotype, whereas birds require estrogens to initiate the female phenotype, and reptiles use both androgens and estrogens for sexual differentiation.

c) Agents that act by endocrine disruption: A number of chemicals have produced abnormal development and/or reproductive function via an endocrine pathway in some species. It is not the purpose of this report to construct an exhaustive list of these. Instead this list will include a few illustrative examples and indicate the classes of chemicals that appear to be of special concern. However, not all chemicals of a given class have similar endocrine-disrupting potential; also, it is likely that chemicals in other classes not mentioned may have endocrine toxicity potential.

Examples of agents that have been shown to alter reproductive development in various species via an endocrine mechanism include:

- Hormones and drugs, including DES, progestogens, androgens, ecdysteroids and farnesyl hormones
- Metabolic inhibitors, including 5-α-reductase inhibitors
- Pesticides, including DDT and its metabolites, chlordecone, and vinclozolin
- Phytoestrogens and mycotoxins
- Other chemicals, including dioxins and some PCBs

These agents do not need to be persistent to have an effect, particularly if the exposure occurs during a critical developmental period. Importantly, prior exposure to persistent chemicals can result in exposure during a critical window of sensitivity even though external exposure had long been terminated.

Several other chemical or chemical classes (e.g., alkylphenols, some phthalates, bisphenol A) have been shown to interfere with some endocrine-mediated processes in some systems, but evidence for effects on reproductive development *in vivo* is lacking. Although these examples suggest direct involvement with steroid receptors, nonreceptor pathways are also potentially important targets.

Last, a discussion of hazard is not complete without mentioning that it takes some level of exposure to have an effect. That is, there is a dose–response relationship for all chemicals. It is important to focus on those chemicals for which endocrine disruption is the most sensitive effect. Agents that have other effects of concern at lower doses can be studied and regulated based on those other effects if they are considered adverse.

d) Modes of action: Modes of action include agonistic and antagonistic receptor binding, and effects on hormone synthesis, storage, release, transport, and clearance. There are many receptor-mediated modes of action, including effects on estrogen, androgen, progesterone, thyroxine, glucocorticoid, and Ah receptors. Others are likely to be identified.

In addition to direct effects on receptors, there are instances of metabolic inhibition and induction that affect steroidogenesis, inhibitors of enzymes that modify hormones (e.g., 5-α-reductase), and effects on plasma transport proteins and neurotransmitter levels (e.g., effects on the hypothalamic–pituitary axis). Differences between species may be marked, and this may be one important source of lack of concordance across species. Last, it will not be a simple matter to predict the action of mixtures.

It is noteworthy that some agents interact with more than one receptor type (e.g., estrogen, androgen, and progesterone receptors) and that there may be multiple mechanisms of action for a single agent, leading to different dose–response curves for different outcomes.

e) Species affected: In vertebrates, there are examples of endocrine disruption from Mammalia (including humans and laboratory animals) to Pisces. Examples in invertebrates include gastropod mollusks (exposed to alkyltin), insects (exposed to insect growth hormones), and crustaceans. It is likely that other examples have yet to be identified. Sensitivity varies by species and population.

f) Species concordance and divergence: As noted above, the level of concordance between species depends on the biological level of organization being examined. The greatest homology tends to occur at the most fundamental levels of organization and less so at the level of the organism. Knowledge of mechanisms of action and of basic comparative endocrinology and embryology will greatly enhance our ability to extrapolate among species.

Although we have considerable information on perturbations of reproductive development as well as on direct effects on the adult, after exposure to chemicals that disrupt the normal functioning of the endocrine system, there remain important uncertainties. In particular the work group noted the following:

a) Species-to-species extrapolation: There is a great deal to learn about basic aspects of comparative endocrinology and embryology. Concerning the former, it is known that birds rely on estrogen for sexual differentiation, whereas mammals rely on androgens, and reptiles utilize both estrogens and androgens. While mammals rely on androgens, it is important to remember that the androgen serves as a precursor for estrogen, which is the effective molecule for sexual differentiation in brain morphology, endocrine secretion, and behavior. Differences have also been observed in the specificity of serum-binding proteins. It is likely that other differences will be found that will help explain the diverse responses to endocrine disruptors.

In comparative toxicology, three factors must be considered. First, there may be pharmacokinetic differences that affect the concentration of the active agent at the target site. Second, there may be pharmacodynamic differences in the interactions of the agent with the molecular target and subsequent responses. Third, superimposed on these are differences in the genetic template across species.

b) Availability and reliability of historical data: Historical trend data are the basis for identifying adverse health effects by interpreting the extent to which the incidence of these effects is changing and/or is associated with other time-related trends. These data are also extremely useful in formulating hypotheses on causation. Therefore, a robust historical database is important for further field and epidemiological research. However, the historical database on human and animal reproductive end points is not adequate for our needs. More prospective and retrospective studies are needed on a

variety of reproductive parameters. Comprehensive evaluation of reproductive parameters in highly exposed populations (for example, sons and daughters of mothers exposed to DES or PCBs) would be useful to identify the most critical effects for which historical trend data should be analyzed. Finally, statistical analysis of historical trend data should be rigorous, as patterns of change may suggest potential underlying causes (e.g., one might suspect different causes for a single cataclysmic decrease in sperm count versus a continuous gradual decline).

c) Effects at low levels of exposure: Effects at low levels of exposure may be qualitatively different from effects at high levels for several reasons, including multiple mechanisms of action, each of which takes effect at a different dose level. Furthermore, an agent may have multiple effects, each having a qualitatively and quantitatively different dose–response curve.

d) Latency: It is known that developmental exposure to an endocrine disruptor can have long latency periods before expression of an adverse effect, the hallmark being DES-induced vaginal adenocarcinoma. Efforts should be made to establish prospective registries of highly exposed populations, such as sons and daughters of mothers exposed to DES, for other latent effects, particularly breast, endometrial, and prostate cancers, as this group is reaching the age when problems become more prevalent. Other latent effects of potential concern include cardiovascular, premature menopause, and altered reproductive behavior in birds and other fauna.

e) Relevance of bioaccumulation and biomagnification: Although it is intuitive that materials which bioaccumulate and biomagnify are of special concern to those species that consume them, the relative contribution of these processes to toxicity is dependent on trophic level in the food web, life stage, physiological conditions favoring lipid mobilization (e.g., pregnancy, lactation, egg laying), and reproductive strategy. More work needs to be done before the contribution of bioaccumulation and biomagnification to the toxicity of a particular agent in a particular species can be fully appreciated.

f) Behavior of mixtures of ligands: Additivity, synergism, and antagonism of components of mixtures acting at the same receptor are complicated by several factors; for example, the interactions may be qualitatively different depending on the concentration and ratio of each component of the mixture and whether they are agonists, antagonists, or combinations. It may be possible to construct a set of rules to explain the behavior of any mixture, given satisfactory knowledge of the activities and mechanisms of action of each component and validation of the system. This approach limits testing to a large but finite number of chemicals in order to characterize virtually infinite numbers of mixture components and concentration. This should be contrasted with the impossibility of empirically testing every possible mixture.

Risk assessment approaches for mixtures also need further refinement. In particular, the toxic equivalency factor (TEF) approach needs improvement. A common mechanism of action must be clearly demonstrated before initiating the process. When calculating TEFs, the lowest experimentally measurable response of the most appropriate effect in the most appropriate species should be used consistently.

g) Basic research: Although much is known about the mechanisms of developmental effects of endocrine disruptors, much more needs to be learned. Two areas that deserve particular attention are improved understanding of dose at the target site and the investigation of additional potential modes of action involving paracrine and autocrine signaling pathways.

h) Sensitive populations: Although we are generally aware of the life stages most sensitive to endocrine disruption, there is still some uncertainty about the relative sensitivity of populations. Marked differences in the responsiveness of different inbred mouse strains to the toxicity of TCDD suggest that there may be substantial variability of response in other species, and for other endocrine disruptors. In wildlife populations, there is uncertainty as to whether differences in susceptibility of subspecies has led to a decrease in genetic variability.

What are the research needs related to the detection of reproductive and developmental effects of endocrine disruptors?

While numerous wildlife and ecosystem pollution studies have assessed reproductive end points and a few well-documented examples of the effects of EDCs in wildlife have been identified, little is known about endocrine-disrupting chemical effects in most wildlife populations. Structured research on the status and trends of populations and communities including natural variations is needed to detect population changes that might not otherwise be apparent until significant losses occur. The group constructed the following list of research needs:

a) Establishing cause and effect relationships: Hypotheses generated from field observations must be tested in the laboratory and in controlled field studies. This will require collection of more complete field data on hormone levels, tissue burdens of chemicals, and more measures of marker expression (e.g., vitellogenin) to guide laboratory studies. Existing long-term ecological field studies should be identified that can be integrated into the endocrine disruptor research agenda to test hypotheses. This research must be multi-disciplinary in nature, and improved mechanisms for information exchange between field and laboratory researchers must be established. Better statistical models to predict risk from observations of exposure and effects are also needed.

b) Biomarkers: Better biomarkers are needed that reflect both exposure to and effects of endocrine disruptors. These biomarkers need to correlate with the most sensitive end points associated with endocrine disruptors. These biomarkers must address species differences, sexual dimorphism characteristics, and be life stage specific. They must be applied in long-term, transgenerational studies to identify biomarkers in offspring that can be measured shortly after exposure and that are predictive of long-term or latent effects.

c) Data collection: More extensive studies are needed of both highly exposed wildlife and human populations (for example, sons and daughters of mothers exposed to DES or PCBs) as well as populations exposed to a contemporary ambient level of endocrine disruptors. Throughout these studies, researchers must consider that there may be no unexposed populations. Research hypotheses should be evaluated in these populations. More information on normal population variation, as well as regional and seasonal effects, should be gathered. Both prospective and retrospective historical trend information is required for wildlife and human populations in order for researchers to identify adverse reproductive health trends (e.g., reduced semen quality and quantity in humans, reduced reproductive success in wildlife) and to develop and test hypotheses about their causation when possible.

More data on exposure monitoring coupled with integrative bioassays of

effects are needed, which would be facilitated by coordination of different agency efforts (e.g., National Oceanographic and Atmospheric Administration [NOAA], U.S. Fish and Wildlife Service [USFWS], U.S. Geological Service [USGS], National Biological Service [NBS]). Traditionally, exposure data have focused on adults. That focus must shift to collecting data for the most sensitive life stages, recognizing that effects may have prolonged latency periods before they are manifested.

d) Basic research: Studies on basic developmental biology need to address the ontogeny of receptor systems. Classical approaches to the study of receptor-based mechanisms may not apply to early developmental periods. Research should be conducted to identify the end points in multigenerational studies that are most sensitive to endocrine disruption. Both receptor and nonreceptor-mediated mechanisms of endocrine disruption should be studied, and the normal hormonal environment of the developing organism and the adult must be better characterized. Better and cheaper analytical tools need to be developed, including molecular probes for gene expression products, and immunoassays for biological tissues. Research efforts would be greatly facilitated by establishment of a repository of radiolabeled compounds, antibodies, and cDNAs available at minimal cost to researchers. Laboratory studies must focus on low-dose exposures reflecting realistic environmental levels, environmental mixtures, and pharmacokinetic parameters, including modeling of nonlinear dose–response relationships.

e) Mixtures: Because little is known about the hazards of chemicals in environmentally relevant mixtures, a scientifically based risk assessment approach is needed to deal with mixtures. Chemical interactions can be very complex and need to be characterized at a number of environmentally relevant dose levels. The model of receptor interaction used in these studies needs to be carefully examined, as a single receptor can activate a number of genes, which is a particular concern with environmental mixtures.

A toxic equivalents approach (TEQ) is potentially useful for assessing the risk posed by multiple chemicals with a common mechanism of action. Validation of this approach should consider multiple variables such as species differences, sensitivity of the end point(s) measured, mechanism of action, nutritional status, co-occurrence of infection, and doses. The most useful approach would use the most appropriate end point in the most appropriate life stage of the appropriate sensitive species.

f) Screening methods: Given the need to test many more chemicals for endocrine-disrupting potential, short-term *in vivo* and *in vitro* tests that are rapid, reliable, and inexpensive must be developed to screen chemicals for relevant hormonal activity. Screening methods will only be useful if they are sensitive to a variety of mechanisms of action by endocrine disruption. The most sensitive end points need to be identified. Behavioral and growth parameters are end points to explore further. A testing approach incorporating both *in vitro* and quantifiable *in vivo* tests that are validated is needed. Where the mode of action is known, research on the molecular basis of the effect of endocrine disruptors on gene expression is needed to identify molecular probes reflecting the morphologic and/or functional changes in the reproductive system. Where the particular mechanism(s) of action of a compound is unknown, more long-term *in vivo* assays are needed. Early life-stage testing in rodents, fish, and amphibians seems promising as an *in vivo* screening method. Quantitative structure–activity relationship (QSAR) models need to be developed further to help prioritize compounds for more extensive testing.

g) Population heterogeneity: The possibility needs to be studied that endocrine-disrupting chemicals may be decreasing the genetic variability of populations through selection pressure. Comparisons of liver tumor frequencies, PAH burdens, age, and length characteristics of brown bullheads collected in the early 1980s from two tributaries of Lake Erie strongly support the hypothesis that the bullheads in the Black River were subjected to an age-selective mortality associated with a high incidence of PAH-associated liver carcinoma (*8*). Genetic diversity estimates for the mitochondrial genome of this species at these and seven other sites in the lower Great Lakes in the late 1980s were always much lower in populations from the contaminated sites than in nearby reference sites (*92*), apparently due to stochastic reductions in population size.

What are the highest priority research needs for reproductive and developmental effects?

Developmental and reproductive toxicity is a problem with important implications for public health and ecosystem health. Research is needed to delineate the contribution of endocrine-disrupting chemicals to the observations of adverse effects on reproduction and development in humans and in wildlife populations. Because of the potential long-term impacts on both individuals and populations, this area deserves a high research priority.

Among the highest priority needs in this area are the following:

a) Controlled laboratory tests of hypotheses generated from field studies.

b) More extensive studies on wildlife and human populations exposed to high levels of endocrine-active toxicants (e.g., the DES and PCB cohorts) to identify the adverse health effects most likely to occur from developmental endocrine disruption.

c) Better definition of normal variability in reproductive parameters and more comprehensive temporal data (both prospective and retrospective) so that potential trends can be identified more readily and reliably, and hypotheses tested regarding their causation.

d) Characterization of the interaction of mixtures of endocrine-active toxicants, and the development and validation of risk assessment methods that adequately account for these interactions.

e) Development of QSAR and short-term screening approaches to identify potential endocrine-active hazards to reproduction and development.

Neurological Effects

What do we know about the neurological effects of endocrine-disrupting agents in humans and wildlife? What are the major classes of chemicals thought to be responsible for these effects? What are the uncertainties associated with the reported effects?

The work group determined that neuroendocrine disruption can be induced by multiple mechanisms. Direct effects on endocrine glands (e.g., the thyroid) may alter the hormonal milieu, which in turn can affect the nervous system, resulting in neurotoxicity. Conversely, EDCs may initially act on the central nervous system (CNS) (e.g., neuroendocrine disruptors), which in turn can influence the endocrine system. It was noted that exposure to chemicals can adversely affect the structure and function of the nervous system without any endocrine system involvement. The group considered several examples of effects produced by disruption of the endocrine system and agreed that alterations in the following would be indicative of neuroendocrine disruption: reproductive behaviors mediated by alterations in the hypothalamic–pituitary axis

(e.g., courtship and parental behavior in avian species); alterations in metabolic rate, which could indirectly affect behavior; altered sexual differentiation in the brain, which could affect sexually dimorphic reproductive and nonreproductive neural end points; and some types of neuroteratogenic effects. The group concluded that there were clear examples in the human and animal literature in which exposure to endocrine disruptors had occurred and effects on behavior, learning and memory, attention, sensory function, and psychomotor development were observed (*60,76,79,93–110*). Some of these effects, however, can also be produced by developmental neurotoxicants having little or no known endocrine-disrupting properties and, therefore, cannot be regarded as specific to the endocrine-disrupting class of chemicals. It was also pointed out that exposure to a number of nonchemical factors (e.g., food or oxygen deprivation, infections, and temperature) could also adversely affect the nervous system resulting in effects similar to those produced by endocrine disruptors. These nonchemical factors may also interact in as yet unpredictable ways with chemical stressors. Therefore, considerable care should be taken to eliminate nonchemical causes before concluding that neurotoxicity is causally related to the effects of a chemical acting on the endocrine system.

The group concluded that there were several examples of chemicals or classes of chemicals that produce neurotoxicity by an endocrine mechanism. It was agreed that environmental toxicologists should consider the dose at which neuroendocrine dysfunctions are produced relative to the concentrations existing in the environment and relative to dose levels at which other toxic effects occur, the relationship between exposure and effect, and the role of naturally occurring chemicals with endocrine-mimicking properties. With these caveats in mind, examples of directly or indirectly acting neuroendocrine disruptors include some PCBs, dioxins, DDT and related chlorinated pesticides and their metabolites, some metals (methylmercury, lead, organotins), insect growth regulators, dithiocarbamates, synthetic steroids, tamoxifen, phytoestrogens, and triazine herbicides. Identification of chemicals as neuroendocrine disruptors should be based on mechanistic information at the cellular or molecular level in the endocrine system or defined functionally in terms of activity on responses known to be mediated by or dependent on hormones. All definitions of neuroendocrine disruptors should be interpreted specifically with respect to gender, hormonal status, and developmental stage, since the expression of toxicities of chemicals may change significantly depending on these variables.

The group identified a number of uncertainties critical to understanding the significance of the effects of neuroendocrine disruptors, including:

a) Chemicals occur as mixtures in the environment, thereby making it difficult to assign cause and effect for specific agents. It is possible that the parent chemical may not affect the endocrine system but is metabolized to an active form. The toxicokinetics of and relative tissue distribution into the nervous system are generally unknown for most chemicals; little is known about the metabolic interaction between chemicals in mixtures.

b) There are ranges of possible specific and nonspecific effects that could be measured. Research to date has used only a small number of techniques and methods, and it is likely that many neuroendocrine effects may be subtle and not easily detected with currently available procedures. It is also a concern that the functions most sensitive to chemically induced alterations in neuroendocrine function are the most difficult to measure in the field.

c) It is critical to know when exposure occurred relative to when the effects are measured. Observed effects could be dependent on a number of extrinsic factors such as seasonal variability and intrinsic factors such as hormonal status. In addition, the nervous system is known to be differentially sensitive to chemical perturbation at various stages of development. A chemical may have a significant effect on neuroendocrine function if exposure occurs at a critical period of development but have little or no effect at other stages of maturation.

d) Several issues related to extrapolation are critical to understanding neuroendocrine disruptors. For example, it is difficult to evaluate the significance to human health of a chemically induced change in a behavior that does not naturally occur in humans, i.e., there are concerns about the appropriateness of some animal models for toxicologic studies. In addition, there are uncertainties about extrapolating from species to species and from experiments conducted in the laboratory to those performed in the field.

e) There are uncertainties about the shape of the respective dose–response curves for many neuroendocrine effects. It is likely that some chemicals may have multiple effects occurring at different points on the dose–response curve.

f) The group concluded that basic information concerning the mechanism of action of chemicals on the developing nervous system and the neurological role of hormones during development would greatly reduce uncertainties about risk of exposure to neuroendocrine disruptors. Furthermore, it is also important to understand the consistency of the effects relative to the hypothesis that chemicals are affecting the nervous system.

What are the research needs related to the detection of neurological effects of endocrine disruptors?

The group considered a number of research needs:

a) Opportunistic field studies: Coordinated epidemiological research to exploit human and wildlife populations, which have known exposures to neuroendocrine disruptors, to define the biological effects most likely to occur:

b) Laboratory/field studies: Systematic field and laboratory studies that focus on critical experimental uncertainties, e.g., dose–response determinations, effects of different duration of exposures, time of exposure during development, and age of assessment and integration of various end points.

c) Mixtures: Systematic research to address the principle of additivity in determining the risk associated with exposure to mixtures.

d) Toxicokinetics: Studies to determine the age group-dependent toxicokinetics and toxicodynamics of environmentally relevant chemicals, with an emphasis on providing better exposure assessments to correlate biological effects with target or tissue dose.

e) Mechanisms of action: Research at the cellular and molecular levels to provide a better understanding of the mechanisms of action for known neuroendocrine disruptors.

f) Basic research: Research to better understand the normal development of the nervous system and the role that endocrine systems may play in that development.

g) Sentinel species and biomarkers: Identification of sentinel species and development of biomarkers of exposure and effects for neuroendocrine disruptors.

h) Identification of sensitive subpopulations: Research to determine if there are

populations or individuals that may be differentially sensitive to neuroendocrine disruptors.

i) Multigeneration assay development: Multigenerational research in both invertebrates and vertebrates to assess the possible transmission of effects across generations

What are the highest priority research needs for neurological effects?

The group agreed that research concerning the effects of chemicals on the neuroendocrine system should have a relatively high priority. Because the developing nervous system is differentially sensitive to chemicals, there is great concern that low-level exposure to environmentally relevant mixtures of chemicals could have subtle, long-lasting effects on nervous system function in a number of animal and human populations. Neurotoxicologic effects have been documented in children exposed to a number of environmentally relevant chemicals, including the PCBs, methylmercury, and lead (*111*), although these do not necessarily act primarily via endocrine-mediated mechanisms. Finally, the group noted that the nervous system interacts with or controls other potential targets (immune and reproductive systems) and serves as an interface between the environment and the internal milieu. It seems likely that effects on other target systems and the manner in which organisms perceive and respond to the environment may involve the neuroendocrine system to some degree. Thus, studies on the mechanisms of neuroendocrine disruption should provide crucial information concerning mechanisms of other biological effects.

Among the highest priority research needs in this area are

a) The initial focus on identifying and documenting effects of concern to humans and wildlife that are possibly mediated by the neuroendocrine system

b) Follow-up studies to determine the parameters of exposure to specific chemicals and the time of assessment of effects on the neuroendocrine system

c) Demonstration of biological plausibility between exposure to chemicals and observed effects in humans and wildlife

d) Studies on the potential mechanism of action of observed effects

e) Attention to the roles of mixture in the effects produced by neuroendocrine disruptors

f) Better understanding of the interaction of the nervous system with other potential targets of endocrine disruptors.

Immunological Effects

What do we know about the immunological effects of endocrine-disrupting agents in humans and wildlife? What are the major classes of chemicals thought responsible for these effects? What are the uncertainties associated with the reported effects?

Published studies have demonstrated associations between autoimmune syndromes and DES exposure (*112*). A relationship is well established between physiological estrogen levels and autoimmune diseases in women (*113–115*). The observations that exposure of humans to DES, TCDD, PCBs, carbamates, organochlorines, organometals, and certain heavy metals alters immune phenotypes or function are suggestive of immunosuppression and potential disease susceptibility (*116–119*). Experimental animal studies support these observations (e.g., *120–126*), although dose–response information is needed to clarify whether these are directly- or indirect-acting agents. With respect to fish and wildlife, it was also noted that several of the agents listed above induce immune suppression or hyperreactivity similar to that reported in experimental animals and humans. Embryonic exposure of trout to aflatoxin has led to alterations in adult immune capacity (*127–129*). With regard to disease susceptibility and exposure, there have been examples such as the dolphin epizootic of 1987 to 1988 (*130*). In this case there was an association with PCBs and DDT in the blood, decreased immune function, and increased incidence of infections among affected individuals (*131*). Impairment in immune function has been reported in bottlenose dolphins exposed to PCBs and DDT (*132*) and in harbor seals fed fish from polluted waters (*133,134*). From 1991 to 1993, specific immune functions and general hematologic parameters were measured in herring gull and Caspian tern chicks from a number of study sites in the Great Lakes chosen across a wide range of organochlorine contamination (primarily PCBs). As the hepatic activity of ethoxyresorufin-*O*-deethylase (EROD), an index of exposure, increased, thymus mass decreased. At highly contaminated sites both gull and tern chicks showed marked reductions in T-cell-mediated immunity as measured by the phytohemagglutinin skin test (*135*).

A variety of immunoassays have been used to demonstrate effects in experimental laboratory animals, humans, fish, and wildlife. These include modulation of antibody responses (both *in vivo* and *in vitro*), the phytohemagglutinin skin test, mitogenesis, phagocytosis, levels of complement or lack of acute phase reactants, cytotoxic T-lymphocyte reactivity, and natural killer cell activity (*136–142*).

Evidence of an increased rate of autoimmunity associated with prenatal DES exposure suggests the possibility that other endocrine-disrupting chemicals (EDCs) may induce a similar pathologic state. Studies are needed to determine if there has been an increase in cases of immune dysregulation in areas or sites where EDC exposures have occurred. Evidence indicates that the incidences of allergy and asthma (which are forms of hypersensitivity) are increasing in humans (*143–145*). It is not known whether EDC exposures are responsible for some part of this development. Alteration of sex–steroid balance has been shown to lead to increased or accelerated onset of autoimmune syndromes in mice (*114*). In rats and mice, heavy metals such as lead, mercury, and gold enhance autoimmune syndromes (*146,147*). There have also been reports of exposures of fish to EDCs in the environment that lead to immune enhancement. Although autoantibodies have been reported in sharks and trout (*148–150*), no attempts have been made to correlate exposure to EDCs with incidences of autoantibodies. In trout, embryonic exposure to aflatoxin B_1 can lead to immune stimulation or suppression in the adult, depending upon the immune parameter analyzed (*129*). Other data suggest that small changes in physiologic levels of estrogens can affect the immune system, and studies in gull and tern chicks in the Great Lakes clearly indicate that the findings are associated with developmental exposures (*135*).

Concerning direct-acting EDCs, although it would appear that these agents directly affect the immune system, it is unknown whether there may be disruptive effects on the endocrine–immune axis. Since the immune and endocrine systems are linked via various cytokine signaling processes (IL-1, ACTH, catecholamines, prolactin, and endorphins), it is likely that EDC effects on the immune system modulate elements of the endocrine or nervous systems or vice versa. Too little is known about the dose–response curves for immunotoxicity, neurotoxicity, or endocrine effects to decipher the independent or interactive effects on these systems. Because of the high degree of intercommunication between these systems, there is a need for

coordinated and cooperative studies among laboratories in all these disciplines.

Although the most forceful arguments for the overall consequences of immune dysfunction would be increased disease incidence, this is difficult to assess in humans or wildlife populations. Furthermore, only certain subpopulations (the very young or elderly) may be affected. Disease may only be manifested as a population decline. Disease trials can be undertaken, but they require controlled laboratory experiments employing populations of wild animals or fish that can easily be maintained in the laboratory. In humans, the variability within a population makes it difficult to decipher exogenously triggered effects.

The fact that employment of a variety of *in vitro* assays has been successful in correlative exposure studies leads to the question of whether those immune parameters can be correlated with the increased risk of disease. A number of immune parameters operate independently (i.e., lysozyme levels, complement activity, phagocytosis, induction of cytotoxic T lymphocytes, plaque-forming cells, etc.). Which combination of these assessments would make for an optimal predictive suite of assays?

Knowledge of normal baseline values for wildlife species, and in most cases humans, is lacking. If these populations are to be screened for perturbations in immune function, control populations must be defined and standardized control values obtained. Also, the types of exposures must be well documented (i.e., dose, length of exposure, timing).

What are the research needs related to the detection of immunological effects of endocrine disruptors?

The work group identified the following research needs related to the assessment of immunological risk to human and wildlife populations:

a) Epidemiological analyses: Human populations with known exposure should receive greater attention, concentrating on the possible association of exposure with autoimmune symptomology, hypersensitivity, and disease incidence. In wildlife populations, particularly marine mammals, analysis must be associative and would require more rapid and inexpensive methods to quantify or identify the presence of EDCs. This will require additional live capture research on marine mammals using noninvasive sampling techniques to establish appropriate baselines of normality. It is especially important to coordinate immunological research activities with reproductive, neurological, and carcinogenesis research. The ability to quickly mobilize groups to address environmental problems, with a tested suite of immunoassays, would be advantageous when new environmental exposures are detected.

b) Mechanisms: Studies on mechanisms of action must be conducted for agents that cause endocrine disruption after initial interaction with the immune system. Studies are also needed that determine the dose level at which immune effects occur secondary to endocrine disruption. Exclusive immune dysfunction at low concentrations would indicate that the primary target of the specific EDC would be the immune system. The endocrine effects caused by many disruptors have not been examined for immune system effects. Studies are also needed to characterize potential effects on the endocrine system of direct-acting immunotoxicants. The general feeling was that many of the endocrine disruptors identified thus far act directly upon the immune system; however, the group did not extensively discuss agents that may affect the immune system via endocrine disruption (e.g., ammonia).

c) Mixtures: Identification of the immunotoxic elements within such mixtures would be a primary aim, although more information would be gained if coordinated studies were conducted between endocrinology, developmental, and neurobiology laboratories. In addition, inexpensive analytical tools are needed to analyze contaminant body burdens. Although there was some amount of uncertainty in the group as to the value of bioassays to grossly quantify classes or groups of EDCs, this avenue might provide an inexpensive means of assessing exposure. However, most samples would be comprised by the presence of mixture and the contributions of agonists and antagonists to the EDC would further complicate analysis.

d) Critical periods: Because studies are lacking on developmental exposure, they will have to be conducted in the laboratory, in long-term human epidemiological studies, or with individually marked wildlife populations. To date, work with agents that have an initial impact on the endocrine system has demonstrated that the most compelling evidence is related to developmentally acquired dysfunction. Furthermore, and perhaps more important, the effects on the developing immune system may be more persistent or longer lasting than those that might occur with adult exposure. The doses needed to elicit these developmental dysfunctions are not as large as those for acute exposures of adults; thus, a much greater percentage of populations may be affected in this way. For some wildlife species, studies on normal developmental immunobiology would have to be conducted prior to any extensive EDC testing on model species.

e) Sentinel species: The choice of model or sentinel species for the assessment of EDC effects on the immune system requires a great deal of forethought. These choices should be well suited to answer specific questions (both of an immunological and endocrinological nature). It was felt that a logical first step might be greatly expedited by agencies such as the U.S. EPA. Their access to information concerning the impact of the environment on populations throughout the United States could provide valuable information for determining potentially good model/sentinel species. These species should also possess the following characteristics: ease of maintenance in the laboratory; ubiquitous distribution in the environment—broad range; accessibility of large numbers; easily bred within a laboratory environment (permits analysis of genetic basis of susceptibility required for developmental analysis and eliminates carry-over environmental effects in dose–response experiments); substantial database on physiologic parameters; inexpensive cost of procurement and maintenance; ecological relevance; short generation time and representative of a large number of species or, at least, groups of species

Upon selection of model species, there should be coordinated optimization and standardization of the immunological assays and their analyses. Major gaps in the endocrine and immune databases of selected species must be addressed immediately. The entire repertoire of sentinel species should account for the variety of environmental conditions that may modulate either the exposure or response to EDCs, including temperature (if an ectotherm), trophic level, and other conditions of the particular niche (i.e., salinity, exposure to sediment, water column, atmosphere.

f) Ecological monitoring: Studies of marked wildlife populations are needed to determine the effects of contaminants on the demographics and to monitor the age and accumulation of EDCs within tissues. Some of the sentinel species used in these studies need to be relatively hardy while others may need to be rather sensitive. For example, trout are often the

first to disappear from a contaminated site, thus suggesting the first indication of a problem. Although this feature in and of itself may be a good marker attribute for a sentinel species, it is also advantageous for the animal to be hardy enough to remain on the site so sampling can reveal the nature of the disorders that occur.

What are the highest priority research needs for immunologic effects?

The work group agreed that the highest research priorities should go to areas where the risk to human and animal life is the greatest. If the impact is on the immune system, the risk to life could obviously be tremendous and would necessitate a commitment to immunotoxicologic research in this area. However, before such action is taken, it was felt that the existence of such problems should be determined by conducting epidemiological analyses of human, wildlife, and sentinel species, as described in the previous section. If risk appears considerable, research should then be directed toward developmental effects as well as toward acute effects, and work should begin as outlined with the most appropriate sentinel/model species.

Among the highest priority research needs are

a) Epidemiological studies in both human and wildlife populations are needed to establish the incidence of immune-related diseases, including immunosuppression, hypersensitivity, and autoimmunity associated with exposure to endocrine-disrupting agents and to determine the impact of these effects on clinical diseases such as infections and cancer.

b) Basic research is required to identify the mechanisms and individual substances that alter the immune system, and to determine whether they act directly or indirectly through alterations of endocrine system function. Included in this research should be determination of dose–response relationships.

c) Methods need to be developed, particularly for wildlife species, to identify and validate sensitive assays that detect immune effects including the selection of appropriate sentinel species. Included in this process should be the development of biomarkers.

d) Studies are needed to determine whether sensitive populations exist. Based upon existing evidence, the immunologic effects of these substances on the very young (i.e., during the developmental phase) are of particular concern.

Risk Methodology Issues

Hazard Identification

Hazard identification includes the collection and evaluation of toxicity data from test systems, epidemiological studies, case reports, and field observations. The number of species evaluated, the number of studies conducted, the quality of the studies, end points evaluated, and other factors are assessed in the context of dosage, route, timing, and duration of exposure. These data are used to determine whether the agent in question poses a hazard, and the context in which it poses a hazard (i.e., is it route specific, species specific, life stage specific, etc.). Discussion focused mainly on prospective hazard detection, whereas from the ecological perspective, retrospective analysis related to post-environmental release of contaminants is often the more important issue. This dichotomy was explored further by the subsequent U.S. EPA-sponsored workshop devoted to ecological issues (*151*).

What are the existing guidelines/testing protocols that evaluate endocrine-related effects?

Ecological test guidelines. The following ecological tests are commonly conducted during the evaluation of industrial chemicals and pesticides. Tests marked with an asterisk offer the opportunity to detect effects relevant to endocrine disruption. It should be noted that the end points of growth and reproduction measured in these tests are apical, and hence possibly reflect effects caused by an underlying endocrine-linked mechanism. However, because of this integration, an explicit endocrine mechanism would not be implicated. The tests include *a*) short- and long-term algal toxicity; *b*) acute and chronic reproduction* tests in aquatic and terrestrial invertebrates; *c*) acute, chronic*, early (embryo, larval*) and full life-cycle* tests in fish; *d*) acute, 14-day, and longer-term reproduction studies*, and egg-dosing studies of hatchabilty and teratology* in avian species; and *e*) acute and chronic effects in plants. Ecological testing uses a small number of surrogate species to represent the environment; therefore, the diversity of reproductive and developmental strategies contained within aquatic and terrestrial organisms may not be fully addressed. It was noted that some of these tests require development of appropriate positive and negative controls and standardization. Furthermore, they typically do not address sublethal effects (e.g., hormone levels, behavior, transgenerational effects on the offspring) often considered fundamental in mammalian testing.

Human health testing guidelines. Various forms of the *a*) 2-year cancer bioassay; *b*) 90-day subchronic toxicity study; *c*) multigenerational reproduction study; *d*) developmental toxicity study; *e*) developmental neurotoxicity tests; and *f*) immunotoxicity tests were discussed. Although these tests are generally used to evaluate the impact on human health, they also play a role in assessing effects in mammalian wildlife species.

It was the consensus of the work group that these tests are intended to detect effects, not to identify mechanisms. For endocrine disruption, the current tests in many cases may fail to determine the appropriate no observed adverse effect level (NOAEL) and may fail to detect the reproductive toxicity of estrogenic pesticides [e.g., methoxychlor (*152*)]. Implementation of the Harmonized Reproductive and Developmental Toxicity Test Guidelines (*153*) from the U.S. EPA will improve the ability of these tests to detect the appropriate NOAELs, including those related to endocrine effects. However, none of the current or proposed test guidelines require the measurement of serum hormone levels. In addition, these tests are poorly designed to evaluate latent effects that result from exposure early in life. It was stated by the work group that the Immunotoxicity and Developmental Neurotoxicity Test Guidelines were not specifically designed to detect the effects of endocrine disruptors on immune function or CNS development, and, although the basic two-year cancer bioassay can and does detect tumors of endocrine organs, transplacental carcinogenesis is not evaluated because the study design does not include exposure during critical stages of development (i.e., prenatal, neonatal, infantile, and pubertal stages of life). It was also noted that current tests generally examine only a single chemical at a time, which does not reflect the real world in which exposure is to complex mixtures of chemicals.

What areas within the present guidelines need refining and improving for the adequate evaluation of effects of endocrine disruptors?

In light of the previously mentioned shortcomings of the current testing guidelines, the work group proposed that research be conducted to develop and validate apical methods to detect endocrine disruptors. Once validated, these methods could be

included in the appropriate test guidelines. Examples of areas where improvements are warranted are as follows:

Ecological tests. Because of the lack of an assessment for transgenerational effects in ecological tests, a number of additions were proposed to the current ecological test guidelines. Research is needed to develop and validate new testing procedures.

a) Avian reproduction studies should include an assessment of growth, viability, fecundity, reproductive morphology, and behavior in birds exposed *in ovo.*

b) Some fish species with a short life cycle may prove to be useful models in which to examine EDCs in the laboratory. They can be studied from prefertilization, through fertilization, hatching, recruitment into the breeding population, and fecundity during adulthood. Their hormonal systems are well characterized and they are susceptible to hormonally induced cancers. Assay methods for hormonal systems of nonmammalian vertebrates need to be validated and made available to field biologists on a much larger scale than currently exists. Additional life-cycle studies for invertebrates as well as fish are needed to provide a more holistic hazard assessment for EDCs.

c) Sexual differentiation studies in invertebrates, fish, reptiles, birds and mammals.

Human health and mammalian tests (also for other vertebrates, as appropriate). *a*) Evaluation of weights, histology, and hormone production (*in vivo* with endocrine challenge tests, or *in vitro* after *in vivo* exposure) of endocrine organs (testis, ovary, thyroid, adrenal, pituitary, etc.) in long-term tests [e.g., (*154*)].

b) Determination of testicular, epididymal, and ejaculated sperm counts.

c) Establishment of landmarks of puberty and reproductive senescence in male and female rats and other species.

d) Assessment of sexually dimorphic behaviors and other CNS functions.

e) Expansion of developmental toxicity studies to include perinatal exposure and a postnatal evaluation of reproduction function of the offspring [i.e., the U.S. EPA Alternative Reproductive Test (ART) protocol (*155*)]. Studies of the effects of EDCs on sexual differentiation have been conducted on members of various classes of vertebrates including, mammals, birds, fish, and reptiles. Some invertebrate species have been examined as well. Such procedures could be used to screen chemicals for EDC activity. While such tests would not be short term, they would be shorter and less expensive than the current long-term rodent studies.

f) Evaluation of the need for transplacental carcinogenesis tests for potent endocrine disruptors, such as DES and TCDD, as well as for less potent phytoestrogens and synthetic environmental estrogens. Examine the utility of short-term tests to predict developmental carcinogenicity, including the methods for estrogenic chemicals (*156–157*).

g) Determination whether mixtures of endocrine-disrupting toxicants act in an additive or nonadditive fashion at low, environmentally relevant exposure concentrations.

Are there, or can there be, developed, short-term in vivo *and* in vitro *techniques to screen toxicants for endocrine disruptor activity?*

While it was acknowledged that the proposed U.S. EPA harmonized test guidelines would detect many or most EDCs, these are long-term, expensive studies, and for these reasons, short-term tests are required to screen more efficiently the large number of environmental agents.

Some participants felt that *in vitro* methods could be used for such screening. However, because large numbers of *in vitro* tests would be needed due to the plethora of mechanisms by which EDCs act (i.e., altering hormone synthesis, transport, receptors, metabolism), there was also strong support for *in vivo* testing. In addition, it is not clear how *in vitro* data could or would be used in the risk assessment process. It was noted that when the *in vivo* concentration of active moieties of an EDC in maternal serum reached the K_i from a receptor binding assay, then all of the pups would likely be severely malformed. Thus, *in vitro* data could be used for risk assessment if additional research indicates that delivered doses in the range of the K_i are associated with adverse developmental effects. It was also noted that *in vitro* potency may not correlate well with *in vivo* toxicity because of mechanistic and pharmacokinetic factors. Many experts in the work group stated that *in vivo* screening systems should be implemented, while others felt that a mixture of *in vivo*, *in vitro,* and QSAR techniques could be used most efficiently to screen toxicants. It was pointed out that screening strategies would vary greatly based on what EDC activity the regulatory agencies decide to screen for (i.e., all mechanisms of action versus a limited subset, like estrogenicity; or all EDCs versus those that cause developmental alterations). The group recognized that these issues warrant further discussion within the scientific community to reconcile the disparity in opinions. The work group discussed selected examples of *in vivo, in vitro* and QSAR methods that might be employed to screen for endocrine-disrupting activity. It was noted that *in vivo* tests are often apical while *in vitro* tests can be more specific. *In vivo* tests are more useful if accompanied by target organ/cell dosimetry of the biologically active moieties. *In vitro* tests also need to determine the actual versus administered concentration of the chemical to account for metabolism, stability, and solubility. Cellular assays must also determine cell viability after toxicant administration. The specificity and limitations of each assay must be clearly defined. Research is needed to develop and validate these assays and to define a testing strategy.

Examples of *in vivo* methods applicable to the detection of endocrine disruption include *a*) rodent models of transplacental carcinogenesis; *b*) sexual differentiation studies in mammals, reptiles, fish, and birds (*in ovo*); *c*) acute and subacute studies of EDCs on endocrine systems (including the hypothalamic–pituitary–gonadal axis, adrenal and other endocrine axes, uterine weight and biochemical responses to estrogens, epididymal and sex accessory gland function, LH surge and ovulation, and pregnancy maintenance including *in vitro* ovarian and placental steroidogenesis); *d*) pubertal alterations induced in male and female rats by EDCs (including landmarks of puberty, serum reproductive, adrenal and thyroid hormones, reproductive organ weights, histology, and *in vitro* hormone production from ovarian, testicular, thyroid and adrenal tissues); and *e*) *in vivo* bioassays in rodent systems (e.g., uterine weight assay (*157*). Clearly, examination is needed of the rich diversity of species within the animal kingdom for useful biomarkers of exposure and effect [i.e., vitellogenesis (158)]. Finally, a comprehensive literature search and further discussion should be conducted to expand the list of existing in *vivo* screening methods used by organizations such as the the World Health Organization and the pharmaceutical industry for the detection of EDCs.

Examples of *in vitro* methodologies applicable to detection of endocrine disruption include *a*) the MCF-7 cell proliferation assay for estrogen and other receptors (*159*); *b*) competitive receptor binding assays for estrogens, androgens,

and progestins (*89,160,161*); *c*) transfected cell assays for hormonal and antihormonal activity (*162–164*); and *d*) *in vitro* alterations of whole and minced adrenal, ovarian, testicular, and placental steroidogenesis or pituitary and hypothalamic hormone production [e.g., (*165*)]. Where possible, attention should be focused on characterizing the difference between developing and adult tissues in these assays.

In addition to direct biological assays, the work group noted that 3-D QSAR models are being developed for ligand–receptor interactions based upon the K_i or IC_{50} data derived from androgen, estrogen, and progesterone competitive binding assays (*166–168*). These data are being used as a training set to develop the QSAR model. Such models are then tested with chemicals with known activity, and when validated, the model can be used to screen libraries of compounds with unknown activities. Similar approaches have been successful for Ah receptor–ligands. Research is needed to validate and expand the training sets of these models for the above steroids; similar efforts need to be initiated for other hormone–receptor interactions. In theory, this approach could also be expanded to include toxicant–enzyme interactions. It was noted that although this technique has incredible potential utility to screen for EDCs, it can result in false negatives with chemicals whose structures lie outside the training set.

Summary of research needs for hazard identification of endocrine disruption.

The emphasis for most of the following research needs is on their application in the context of identifying the effects of EDCs in developing organisms. They are

a) Additional validated end points to supplement current test guidelines and expand the availability of hormonal assay, especially for nonmammalian species.

b) Tests and biomarkers to identify acute and latent effects such as testicular and prostatic cancer, premature death, shortened reproductive lifespan, etc.

c) Transplacental toxicology studies for cancer and noncancer end points.

d) *In vivo* and *in vitro* studies of complex mixtures (TEFs for EDCs).

e) Expanded developmental toxicity studies to include postnatal observations.

f) Measurement of target organ dosimetry *in vivo* to determine if adverse effects can be predicted from *in vitro* IC_{50} or K_i values.

g) Expanded training set data for current QSAR models and development of new models.

h) Correlation of aquatic and wildlife models with mammalian models.

i) Better coordination of research among multidisciplinary labs.

j) Improved links between laboratory and field studies to test hypotheses.

k) Examination of multiple end points as well as performance of multiple tests on EDCs.

l) Critical review of additional short-term *in vitro* and *in vivo* tests to screen EDCs in terms of cost, ease of implementation, specificity, and limitations.

m) Continued discussion on the development of short-term *in vitro* and *in vivo* tests because a complete assessment of endocrine disruption likely will require a battery of *in vitro* and *in vivo* tests. Such information will facilitate the use of mechanistic data in risk assessment.

Dose–Response Assessment

Throughout the discussions on human and ecological effects of endocrine disruptors, the workshop participants consistently agreed that timing of exposure was critical to the understanding of dose–response relationships. This is true for the effects of cancer as well as for the developmental, reproductive, immunologic, and neurological effects. Numerous examples were discussed, including hormonal influences on breast cancer where age at exposure is a known risk factor. Similarly, endocrine disruption of the developing brain can permanently alter behavior, whereas similar exposures to a fully differentiated brain could be without effect. Ecological and wildlife effects are also strongly influenced by the timing of exposure (e.g., during the breeding season). Research is critical on how timing of exposure to endocrine disruptors influences dose–response relationships.

Before addressing the specific questions presented to the work group, it is worthwhile to review some of the general recommendations targeted at filling knowledge gaps that create uncertainty in dose–response evaluations. These overarching issues include:

a) Risk assessment issues should be explicitly considered when studies are designed for health or ecological effects. Of particular relevance is the issue of dose selection. Ideally, the doses used should span a wide range to identify both toxic and mechanistic end points, and there should be sufficient numbers of animals and range of doses to track the various end points.

b) While there may never be complete knowledge on the mechanism(s) of action for any chemical, some knowledge on key events could be sufficient to justify the use of mechanistic information in dose–response evaluations.

c) Mechanistic information is most useful when it is linked to adverse outcomes in cases in which several discrete events (molecular and biological) are part of the mechanism of action. Within technical and economic limits, dose–response information should be obtained on as many relevant events as possible. Identification of mechanistic information on the rate-limiting steps in the induction of toxicity is a most useful outcome of this line of research.

d) Population heterogeneity needs to be characterized to improve risk assessment decisions. For human health, a number of factors contribute to a wide range of risks, including genetic predisposition, age (embryos, fetuses, and children are not just small adults), gender, diet, disease conditions, and past exposures. The range of risk modulators may be even greater for complex ecosystems but little information is available in this area.

e) Effects of endocrine disrupters on ecological and human health have both distinct and common features. Studies to identify their common features were strongly recommended by the dose–response work group.

f) Evaluation of the health and environmental effects of endocrine disruptors will be most credible when information is available at several levels such as toxicity, studies, mechanistic and epidemiological studies, and field studies. Well-planned and coordinated multidisciplinary studies are encouraged.

g) Increased reliance on biology and mechanisms will create an increased need to reevaluate potential risks as has occurred with dioxin.

h) Information exchange systems for the scientific, regulatory, medical, public interest, and community sectors need improvement. The Internet affords an excellent opportunity to disseminate relevant information to a broad audience.

What are the existing test guidelines and methodologies used to evaluate endocrine-related effects? Are there areas within our present guidelines that need to be refined and improved for the adequate evaluation of dose–response effects of endocrine disruptors?

There are no specific guidelines to estimate dose–response relationships for endocrine disruptors. Endocrine disruption is inferred

from results from studies such as those on cancer or reproduction. The work group felt that such inferences of endocrine disruption should lead to experimentation (toxicity, mechanistic, epidemiological, field studies) designed to characterize endocrine activity.

General recommendations for experimental studies. The work group recommended an aggressive program of improving experimental design that should emphasize the following:

a) Doses should span a wide range, including environmentally relevant doses for human, wildlife, and ecosystem exposures. A sufficient number of doses should be used to characterize dose–response relationships for each relevant end point.

b) Studies should quantify and evaluate multiple end points. End points should be mechanistic and biological and should be based on existing knowledge about the structural properties and effects of the chemical being studied.

c) End points should be quantified and linked in the same animal or ecosystem, to the extent possible, as well as across species and ecosystems to facilitate the development of biologically based models for estimating dose–response relationships.

d) The use of expanded protocols should be selective and based on resources, mechanistic knowledge, and risk assessment needs.

General recommendations for human studies. The work group felt that there are opportunities to use existing human data and to conduct new research to improve risk assessments for endocrine disruptors. Specifically, the work group recommends that

a) Existing records on occupational and medical exposures to putative and known endocrine disruptors should be scrutinized for associations with altered incidences of adverse health effects.

b) It would be helpful if consensus could be reached on the most useful questions to be included during medical examinations.

c) Biomarker studies need to address comparative responses between experimental systems and humans.

d) Heterogeneity in dose response, based on genetics, age, gender, and nutrition, needs to be characterized.

Are there unifying dose–response concepts for endocrine disruptors (i.e., threshold, linear, sublinear)?

The work group unanimously felt that a common dose response for all effects and for all endocrine disruption should not be expected. This conclusion was based on the many different kinds of hormonal actions of chemicals categorized as endocrine disruptors. These activities include estrogenic, antiestrogenic, antiandrogenic, growth factor modulation, cytokine modulation, modulation of hormone metabolism, and many others. The conclusion was also based on the knowledge that there are several steps in hormone action and that different environmental agents may intervene in different processes.

Moreover, there is considerable cell specificity in hormone action such that the same hormone and the same receptor can produce quantitatively and qualitatively different responses depending on cell type, age, and other factors. The diverse mechanisms of hormone action are an active area of research; it appears that factors such as receptor number, DNA response elements, signal amplification, desensitization, interactions with transcription factors, ligand metabolism, and the presence of cellular agonists could significantly modify dose–response relationships for any given endocrine disruptor (*169,170*).

What are the research needs in the evaluation of dose–response relationships?

The work group felt that research on mechanisms of hormone action could have spin-off benefits for improving evaluation of dose–response relationships. For example, the sensitivity of cells, tissues, or developmental stages to an environmental hormone could be predicted with improved accuracy. Also, it would be helpful to model common steps in hormone action such as ligand–receptor interactions with responsive genes. Additionally, chemicals that share common pharmacologic properties may share a common response model. The work group cautions, however, that endocrine disruption may occur through mechanisms other than binding to cellular receptors (i.e., inhibition of enzymes of steroid hormone metabolism). The existence of multiple mechanisms both complicates evaluation of dose–response relationships and offers opportunities to better predict low-dose effects for different kinds of mechanisms of endocrine disruption. In addition to studies of known receptors, there may be unknown receptors, including orphan receptors, on which new research is needed.

There was considerable discussion of knowledge gaps in mechanisms of hormone action, as these gaps create uncertainty in the evaluation of dose–response relationships for endocrine disruptors. Although a great deal is known about the initial steps in hormone action such as binding to receptors and transcriptional activation of responsive genes, very little is known about how those changes in gene expression lead to biological effects such as cell proliferation. This lessens the credibility of biologically based models for predicting dose–response relationships for endocrine disruptors. However, several work group members felt that such models still represent an improvement over the default approaches currently used in risk assessments. For example, it may be possible to compare potencies of chemicals apparently acting through common mechanisms (i.e., binding to a specific receptor) and to better predict targets or potentially sensitive cells or tissues. It is also important to distinguish steps in the mechanism that can alter the slope of the dose–response curve, potency, or target organ specificity.

The role of interactions between endogenous and exogenous hormones was discussed. This led to the recommendation that baseline data on endogenous hormones, including cyclicity and differences in tissue concentrations, are needed not only to improve the ability to predict the environmental or health consequences of endocrine disruptors but also as input into mathematical models of development and endocrine system function.

Finally, issues related to the dose–response assessment of mixtures were addressed. Considerable debate exists regarding about dose–response relationships for single compounds even when a reasonable amount of toxicologic data is available. Yet humans and ecosystems are exposed to a vast array of chemicals that may interact by potentiation, synergism, inhibition, and antagonism of toxic effects. Although progress will be slow in risk assessment of mixtures, the following recommendations were developed:

a) Include environmentally relevant doses and ratios of chemicals in ecological, wildlife, and experimental animal studies

b) Use the knowledge of mechanisms and appropriate biomarkers (sensitive and specific) to dissect major contributors to toxicity

c) Determine the pharmacokinetics of toxic chemicals, within mixtures, and the dose at target tissue

d) Improve TEF estimates for environmental agents that interact with specific endogenous receptors (e.g., Ah and estrogen receptor ligands) by systematically testing

assumptions inherent to the TEQ approach. These assumptions include the similarity of mechanism and the role of persistence and accumulation. The work group also felt that TEF estimates would be improved by careful selection of experimental systems used to determine relative potencies and the use of appropriate dose ranges, including low doses to separate direct from indirect effects on the endocrine system.

Are there particular elements to be considered in dose–response evaluation in wildlife species?

The work group felt that evaluation of dose–response relationships to assess ecosystem and wildlife effects will be extremely difficult but would benefit by implementation of the following considerations:

a) Improved understanding of the factors, such as genetic diversity, that are critical to the maintenance of integrated ecosystems. It will be difficult to characterize dose–response relationships without improved knowledge of normal fluctuations in ecosystem constituents.

b) Laboratory and field studies need to be better coordinated. In addition, better definition is needed of what constitutes field validation of a laboratory finding as well as laboratory verification of an effect observed in the field.

c) Guidelines need to be developed for selection of sentinel species and evaluation of dose–response relationships. Guidelines for dose–response assessment should be general and not overly prescriptive.

Is it feasible to develop complete biologically based dose–response models for endocrine disruptors?

The work group encouraged the development of biomathematical models for endocrine systems, recognizing that there will not be a single model applicable to all hormones. However, the shared steps for some systems (e.g., hormone receptor binding) could be modeled using the same set of equations. These models should incorporate data from humans, rodents, and the ecological species. Development of these models must include time dependence of hormonal action.

Statistical and mathematical models for toxic end points such as cancer, reproduction, or development should be helpful in identifying knowledge gaps that create uncertainty in dose–response estimates and, in this way, can focus available resources in the most productive way. These efforts will require increased resources for fostering the multidisciplinary research needed for the development of models for health and environmental effects. Establishing cross-disciplinary programs for mathematical and simulation training for biologists and biological training for statisticians and mathematicians would enhance the credibility of biologically based models for endocrine disruptors.

Exposure Assessment

What chemicals in the environment are of concern for endocrine disruption?

The work group agreed that developing a comprehensive list of putative endocrine disruptors would take longer than the time available at the workshop. Any list so constructed would not be entirely accurate or comprehensive at this time. Nonetheless, from the viewpoint of exposure assessment, it was agreed that EDCs should be categorized as follows:

Use pattern—for example, herbicides, insecticides, fungicides, hormones, etc.

Chemical structure or class—for example, dioxins, halogenated biphenyls, alkyloxyphenols, etc.

Biological function and mechanism of action—for example, estrogen mimic, androgen inhibitor, etc.

What do we know about the status and trends of putative EDCs?

The initial discussion focused on what we know and what we need to know about the status and trends of putative EDCs. Environmental and tissue levels of some EDCs, such as DDT and its analogs, and PCBs, have declined in some countries and in most areas of the United States in response to regulation (*171–178*). Uncertainty still exists regarding future trends of these compounds, however, because of offshore inputs and releases from stored materials. It also appears that for many EDCs, the environmental concentrations that declined from the mid-1970s through the early 1980s have now reached a plateau (*179,180*). This is a cause for concern. For most other EDCs, particularly new chemicals or chemicals that have not been routinely monitored, the trends are unknown.

The information needs regarding status and trends of EDCs are considerable. A better understanding is needed of the fate and transport of new and existing chemicals, particularly among the different environmental compartments (water, sediment, biota). Key concerns have been raised about air and water serving as transport media and exposure routes for EDCs for both humans and wildlife. The importance of water use practices and how they impact on exposure must be more thoroughly investigated. Water use practices that contribute to EDC exposure, such as agriculture, sewage discharge, unfinished waters, and unregulated drinking water sources, require review. Any research on water use must consider factors that affect flow and dilution such as season, diversion, and regulated release of impounded waters.

Exposure assessment, particularly as it involves human health, must focus on vulnerable groups, both in terms of life stage and lifestyle. Exposure assessment for the critical development stages is a high research priority. This includes pregnancy, gestation, lactation, adolescence, and senescence. Vulnerability of different groups in the population will be affected by lifestyle factors such as subsistence hunting and fishing and avid sportsmen who consume fish and wildlife, or host factors such as metabolic differences among polymorphic groups, special dietary habits, and age (e.g., the types and rates of food consumption in children). While the work group agreed that diet would likely be the major exposure route, an approach based on integrated exposure assessment needs to be taken. All routes should be examined (e.g., dermal, inhalation, and ingestion). The work group stressed the importance of a global perspective on exposure. EDCs that may have restricted or no use in the United States are still used in other countries and may become sources of exposure through either imported food or atmospheric transport and deposition. Further, the potential for human or wildlife exposure to multiple chemicals that may function as EDCs should be included in any exposure assessment.

The most critical need on status and trends is for the continuation and improvement of monitoring of the environment for the presence and magnitude of contaminants. Existing programs that furnish repeated measures of chemical contamination in the environment or in food provide our only indication of whether exposure is increasing or decreasing, and to what magnitude. Therefore, the work group strongly recommended continuation of existing programs such as the National Human Health and Nutrition Examination Survey (NHANES) (*181*); the National Human Exposure Assessment Survey (NHEXAS) (*182*); the Market Basket Analysis of the U.S. Food and Drug Administration; the Pesticide Data Program of the U.S.

Department of Agriculture; the National Contaminant Biomonitoring Program of the National Bureau of Standards (*173,183*); and the NOAA Status and Trends Program (*184*). Existing international monitoring efforts must be continued. For new programs or for improving existing programs, the work group felt analytical expenses could be reduced through specificity of analysis. Where markers or bioassays are able to replace chemical analysis, they should be used, and analytes should be targeted to meet the specific monitoring need of the designed program.

More research needs to be focused on development and validation of monitoring tools. Current results with caged organisms have been successful because they provide biologic relevance to the estimation of exposure. Despite the drawbacks inherent in using caged organisms, such as stress associated with captivity and the absence of exposure during the sensitive reproductive and developmental stages, the work group felt this tool warranted further development. Research also should continue on other monitoring tools such as *in situ* samplers that mimic biological tissue, or assays used with field grab samples. As field tools to measure exposure assessment become more available, concurrent research in toxicity identification evaluation (TIE) procedures (*185*) must occur to enable verification of causative agents.

What are the assumptions in estimating exposure to endocrine disruptors?

Valid exposure assessment requires that certain assumptions be either accepted or proven. The work group assumed that environmental concentrations did not equate to exposure because of several factors. Activity patterns such as migratory behavior, bioavailability of compounds, and lifecycle stage are examples of factors that limit the use of environmental concentrations. Body burdens may be much better indicators of exposure, especially for persistent chemicals, but they also may vary seasonally and with age (*186*). However, research is needed to develop tissue-specific body burdens and determine how they translate to specific dose. This will require full understanding of the physiology and toxicokinetics of the compound and its host. Assessing bioavailability is complicated by transplacental boundaries, interaction with transport molecules, and matrix binding (e.g., sediment particles, dissolved organic carbon) and should be a focus of research.

Another critical assumption can be made based on knowledge of the actions of EDCs; that is, a population of breeding adults with apparently healthy offspring, or young in the population does not necessarily indicate health. The ability of some EDCs to evoke transgenerational effects requires a more in-depth look at the health of a population. Reproductive capacity and normal reproductive functioning of the offspring may be the most sensitive test of population health. Research is needed to develop preclinical indicators of transgenerational impacts so that they can be linked to strategies that limit or eliminate exposure.

To what degree can body burdens in the general populations of humans and wildlife be identified by exposure or host factors?

One of the research needs that was identified and given the highest priority was the further development and validation of indicators of exposure to EDCs. Whether these indicators are biomarkers or bioassays, they must be sensitive and specific to both persistent and nonpersistent compounds. These indicators must be validated on a species-by-species basis. Examples of some measures that have been applied include vitellogenin production in male fish (*158,187*) and induction of cytochrome P450 (*188*), although in both instances, lack of response does not necessarily equate to lack of exposure.

In addition to bioindicators, other indices that may be termed population measures need to be applied to EDC exposure. Sperm counts, sex ratios, and incidence of a specific tumor or abnormality are examples (*74,189–191*). If these prove to be indices of effect, changes in these population measures could be used to identify populations potentially exposed to EDCs. Exposure indicators then could be used to quantify the dose–response relationship. The work group proposed a scenario in which indicators could be used in a tiered hierarchy that would demonstrate evidence of exposure to EDCs; clarification of the mechanism of effect (e.g., estrogen mimic, androgen inhibitor); and TIE methods to identify responsible compounds. Given these needs, indicators should be sensitive at ambient levels of contamination and specific to chemicals and end points that would enable identification of mechanism of action.

Are there specific confounding factors or temporal patterns of exposure to be considered in the assessment of public and wildlife health implications of endocrine disruptors?

Estimating the exposure of humans and wildlife to potential EDCs creates a unique set of confounding factors. While this list is certainly not comprehensive, the work group identified the following significant factors:

a) Time lags between exposure and effect: The transgenerational nature of some EDC effects may be the single most complicating factor. All of the potential latent effects that may occur from short-term exposures during critical developmental windows have not yet been identified.

b) Seasonality: Because of the sensitivity of reproductive stages to EDCs, seasonality will be extremely important to wildlife. In addition, the association of EDCs with the aquatic environment is complicated by seasonal rainfall, storm runoff, and water releases.

c) Species variability: Perhaps this is no more important for EDC exposure than with any other toxicant effect. However, the work group particularly stressed that more basic research is needed on general endocrine physiology of target species.

d) Multiple chemical exposures: This, too, is a confounding factor for any toxicant. It is especially identified here because of the potential for joint toxic action and the presence of naturally occurring phytoestrogens.

A comprehensive list would identify a number of general confounding factors such as past exposure history, occupational exposures, nutritional status, trophic structure and other relevant factors.

Summary of research needs for exposure assessment.

A number of research needs were identified in the previous section. The following list was deemed to be of the highest priority:

a) Monitoring: Maintain and increase monitoring efforts that help identify status and trends of EDCs, including chemical monitoring programs and population measures such as wildlife reproductive success, sperm count studies, etc. These programs are critical to the identification of populations at risk.

b) Biomarkers: Develop and validate biological indices for use as screening tools. More biomarkers, bioassays, and population measures are needed that are sensitive at relevant environmental concentrations and are mechanism specific.

c) Exposure hierarchies: Develop an applied, integrative iterative hierarchy of exposure indicators. Components should range from screening level tests to much more predictive indicators. This approach should be designed to avoid false negatives in initial tiers and progress in final tiers to

more predictive indicators that are linked to effects.

Risk Characterization

The National Academy of Sciences' Risk Assessment paradigm was used as a tool to identify research needs. In this case, the approach had limited success since the majority of the group members had limited experience with the specific composition of a risk characterization. The work group, however, did identify several major points for which there was broad agreement.

Normal functioning of the endocrine system encompasses a wide fluctuation of hormone and other biological indices, which reflect among other things, circadian rhythm, season (temperature, light), age, and gender. For example, concentration extremes of sex hormones occur at specified times for normal physiologic functions; such periods include sexual differentiation, puberty, reproductive cycles, parturition, lactation, and menopause. The presence and magnitude of certain hormones are critical to normal ontogenic development, including some neurobehavioral programming, in a broad range of biota that include insects and other invertebrates, amphibia, reptiles, fish, birds, and mammals. Studies in wildlife and in the laboratory have demonstrated that certain chemicals may perturb specific endocrine functions at specified periods during the life span. The consequences of exposure during these windows of sensitivity may manifest themselves at later periods of life. To better understand and predict specific circumstances under which adverse effects may occur, it is essential that there be better identification and characterization of these critical exposure windows. The homology of these sensitive periods across species is also important for accurate risk prediction.

To understand the nature and degree to which an adverse effect reflects response to a nonendogenous chemical with endocrine properties or alters the type and magnitude of endogenous hormones often requires consideration of a broad range of biologic interactions. For example, the role of agents from environmental sources such as phytoestrogens or estrogenic products from fungi should be considered. It was recommended that the current literature on phytoestrogens be reviewed as a prerequisite for determining additional data needs in this area. A comprehensive bibliography for phytoestrogens is available on the Internet (unpublished data).

The traditional risk assessment paradigm can be used to adequately identify and characterize chemicals that cause adverse effects by altering endocrine processes. However, the assessment paradigm should be tailored to permit consideration of such factors as normal fluctuations of endogenous levels of hormones, impact of other agents (phytoestrogens), the existence of critical windows of sensitivity, and the need to understand the significance of subtle effects at low doses. In other words, dose effect may need to be expressed in several contexts such as adult effects, adult dose effect in a particular physiologic state (such as immediately postpartum or during lactation), or at a particular window of sensitivity for causing developmental toxicity.

Endocrine effects often occur subsequent to receptor–ligand binding. A variety of agents may interact with a binding site acting as agonists, partial agonists, or antagonists. Since exposures may entail simultaneous interaction with various endogenous and exogenous substances, it is critical that receptor theory be used to develop or refine quantitative models for estimating the effects of such exposures.

The limited utility, to date, of SAR to predict biological effect for estrogenic agents was noted. Perhaps a useful criterion for screening EDCs for more robust study is whether they can elicit (or inhibit in the case of antagonists) a transcriptional event. Such a criterion would only serve to screen out agents; agents that met this sort of functional definition would still need to be characterized before determining if they have actual toxicological potential. However, such tests would still be limited in their abilities to detect the action of metabolites.

The presumed receptor-based mechanisms, responsible for at least some adverse endocrine-modulated effects, present a unique opportunity to establish a common biologically relevant risk assessment process for all effects, i.e., developmental, immunologic, neurological, and carcinogenic effects. The group is not aware of a biological basis for selecting different models to quantitatively estimate cancer or noncancer effects for chemicals that act by endocrine-mediated mechanisms.

Summary Recommendations

The majority of the participants at the workshop agreed that the endocrine disruptor hypothesis was of sufficient concern to warrant a concerted research effort. In particular, the study of potential effects on the development of reproductive capability at multiple phylogenetic levels was deemed the most important area in need of attention. It was repeatedly emphasized that the developing embryo, fetus, and neonate should not be viewed as small adults and that the processes of development are especially vulnerable to brief periods of endocrine disruption. However, for many of the effects reported in both wildlife and humans that have been attributed to, or associated with, endocrine disruption, exposure assessment has generally been inadequate for quantitative risk assessment. Because of this, some participants felt it was difficult to critically evaluate and establish the level of priority relative to other research topics. Still other participants reminded the work group not to lose sight of the presence of naturally occurring endocrine disruptors (e.g., phytoestrogens) as the effects of man-made chemicals are studied.

Several general comments emanated from the discussions. These include the recognition that there was a great advantage in bringing together a multidisciplinary group of scientists representing both the human health and ecological health viewpoints to help identify common issues and that this interaction must be nurtured as the research agenda unfolds. The work group noted that some key similarities and differences exist between endocrine disruptors and other chemicals that can cause adverse biological effects. Two of the key differences are the presence of natural ligands within the body that must interact at some level with the exogenous chemical; and that the concentrations of the natural ligands within the body fluctuate during the life cycle and must be maintained within narrow limits at key times during development. This latter point indicates that timing of exposure is a very significant factor in any assessment. Last, the mechanistic basis of the interaction with biological systems presages the induction of subtle effects at low doses that must be interpreted as to whether or not the effects are adverse. As the level of organization at which biologic responses to endocrine disruptors are observed decreases (e.g., from physiologic to cellular to molecular), the challenge to describe the effects as adverse at the level of the individual and the population increases. In this regard, endocrine disruptors are not unlike other types of chemicals for which toxicologic information is amassed.

In general, it was felt that linking specific exposures to specific effects in the general environment would often be difficult because of the complexities of exposure,

the latency of the effects, and, at times, the subtle nature of the outcomes. Therefore, confirmation of the validity of the hypothesis will rely heavily on application of the Hill criteria (*192,193*) for causality (strength of the association, presence of a dose–response relationship, specificity of the association, consistency across studies, biological plausibility, and coherence of the evidence). Such considerations will have significant impact on the types of research activities necessary to adequately confirm or refute the central hypothesis. A composite representation of the identified needs is provided in Table 2. Ten broad categories of research needs were identified: basic research, biomarkers, database development, exposure determination, exposure follow-up, hazard identification, mixtures, multidisciplinary studies, risk assessment models, and sentinel species. Within each of these categories, the work groups that identified each need are indicated. The neurological, immunological and carcinogenic work groups all noted the complexity of identifying whether effects of xenobiotics on those systems were the result of primary or secondary aspects of endocrine disruption. This concept is developed in Figure 1, which portrays not only the interaction between the endocrine system and the target organs but also the interactions among the target organs themselves. For these reasons, identifying agents as direct or indirect endocrine disruptors is problematic, and necessitates research to

Table 2. Composite research needs identified by the eight work groups.

Research area	Work group origin[a]	Research need
Basic research	R, ***N***, ***I***, DR	Understanding the cellular and molecular mechanisms, including nonreceptor mechanisms, for EDCs
	R	Sensitive, inexpensive, and widely available analytical tools
	C	Animal and cellular models of endocrine-mediated tumors
	R, N, DR	Ontogeny of receptor-based systems and role in regulating development
	DR, RC	Understanding of the mechanisms and biological significance of subtle low-dose effects
	RC	Identify and characterize critical windows of susceptibility across species
	DR	Characterize source of population heterogeneity in responsiveness (age, gender, nutrition, etc.)
Biomarkers	***C***, R, N, ***I***, DR	Development of biomarkers of exposure and effects of EDCs
	EX	Develop and validate biological indices as screening tools for exposure assessment
	HD	Development of biomarkers for latent effects
Database development	***R***	Information on normal population variation, regionnal, and seasonal effects
	HD	Critical review of short-term tests for EDCs
	R	Prospective and retrospective reproductive health trends
	R	Field data on hormone levels, body burdens, and gene expression markers
	C	Systematic potency comparison of endogenous versus exogenous substances
	C	Surveillance systems for cancer incidence and mortality in wildlife
Exposure determination	I	Rapid and inexpensive exposure monitoring methods for use in wildlife populations
	EX	Increased monitoring efforts to identify status and trends of EDCs
Exposure follow-up	I	Multidisciplinary teams to study exposed populations
	I	Autoimmune symptomology, hypersensitivity, and disease in EDC-exposed humans
	C, ***R***, ***N***, ***I***	Coordinated research on exposed humans, wildlife, and sentinel species
Hazard identification	HD	Target organ dosimetry for comparison with ligad-binding affinities
	R, ***HD***	Expanded development of QSAR models for hazard detection and ranking
	RC	Identification of transcriptional events after ligand binding as QSAR input
	R, ***HD***	Development and validation of apical methods to detect EDCs
	N, I, HD	Perinatal and multigenerational exposure toxicity studies for cancer and noncancer effects
Mixtures	***N***	Research to address the additivity principle for mixtures
	R, HD, ***DR***	*In vitro* and *in vivo* studies of complex mixtures to evaluate validity of TEFs
	R, I, ***DR***	Identification and testing of environmentally relevant mixtures
	C	Systematic evaluation of species-, cellular-, and age-dependent response to mixtures of EDCs
Multidisciplinary studies	***N***	Systematic field and laboratory studies focused on critical uncertainties
	R, HD, DR	Laboratory–field hypothesis-based studies and improved information exchange
	HD	Examination of correlation of effects between wildlife and mammalian models
	N, I, DR	Multidisciplinary studies on effects of endocrine disruption
	HD	Examination of multiple end points and multiple tests of ED action
Risk assessment models	R	Statistical models to predict risk from exposure and effects
	DR	Improvements in study design (dose selection, end points, end point linkages)
	EX	Development of applied integrative iterative hierarchy of exposure indicators
	C, ***DR***	Evaluation of toxicity and mechanistic end points across species common steps and chemical classes)
	N	Toxicokinetics and toxicodynamic studies of environmentally relevant chemicals
	DR, ***RC***	Quantitative dose–response models based upon receptor theory and biochemical interactions
	DR	Establishment of training programs in biomathematics for BBDR model construction
Sentinel species	C, N, ***I***, DR	Identification and monitoring of differentially susceptible sentinel species
	C	Cancer studies in domestic animals and pets

[a]BBDR, biologically based dose–response model; C, carcinogenesis; R, reproductive toxicity; N, neurotoxicity; I, immunotoxicity; HD, hazard detection; DR, dose response; EX, exposure assessment; RC, risk characterization. Designations in bold and italics were among the highest priority needs identified by the respective work groups. In general, carcinogenic and reproductive studies were considered to be higher priorities for biological effects research, while exposure assessment was a recognized deficiency in most population studies.

carefully define dose–response relationships across multiple end points, to delineate the proximate mechanism of action, and to ascertain the complete organismal response to an environmental exposure. However, in terms of protection of human or wildlife populations, it is less important to determine whether effects are primary or secondary once they are observed in the general environment.

Workshop participants were in general agreement that the highest priorities for biologic effect research lie in the areas of development of reproductive capability and carcinogenesis, as these end points have had greater documentation of being adversely affected by alterations in endocrine function than have either the nervous or immune system. However, the incidence of effects on the nervous and immune systems may be underestimated at this point because of incomplete characterization of the biologic effects of endocrine disruptors. In addition, it should be noted that many reproductive effects, especially those involving a behavioral component, are mediated by effects on neuroendocrine function. In particular, identification and characterization of effects on the developing reproductive system were considered to be high priority for additional research because of the high sensitivity and frequent irreversibility of effects after even brief exposures. More refined exposure assessments and research on the toxicology of mixtures were also considered to be of great importance. Special emphasis was placed on the unique challenges endocrine disruptors might pose to the risk assessment paradigm. Interestingly, understanding the basic mechanisms of endocrine disruption induced by various chemicals was seen as an advantage in that this knowledge may result in a common, biologically based, risk assessment process for all effects (i.e., both cancer and noncancer).

At least three outcomes are expected from the workshop. The first of these is the publication of this report in the scientific literature so that it is readily available to both researchers and the public. Second, within the U.S. EPA, the Office of Research and Development in conjunction with input from the Program Offices will be developing an augmented research initiative beginning in Fiscal Year 1996 to implement some of the recommendations of the workshop. To assist in further focusing the research needs for endocrine disruptors for research supported by the U.S. EPA, a workshop on ecological research needs was held in Duluth, Minnesota, in June 1995 (*151*). Persons seeking information from that effort should contact Dr. Gary Ankley of the MED/NHEERL/USEPA in Duluth. Finally, an Endocrine Disruptor Research Coordination Workshop has been formed under the auspices of the National Science and Technology Council that will *a*) develop a federal research strategy that addresses the key scientific uncertainties for endocrine disruptors, *b*) inventory related ongoing federal research efforts, *c*) identify research gaps between ongoing research programs and needs identified in the strategic plan, and facilitate cooordination and cooperation across the federal government to address them, *d*) initiate outreach efforts to engage public interest, private sector, and international groups with interest in this issue, and *e*) promote educational activities such as symposia and workshops to disseminate endocrine disruptor information across the scientific community. Persons wishing to find out more about this effort should contact the senior author of this report.

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