SCIENTIFIC COMMITTEE ON PROBLEMS OF THE ENVIRONMENT

AND

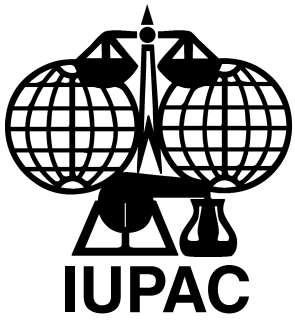
INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

Implications of Endocrine Active Substances for Humans and Wildlife: Executive Summary

J. Miyamoto and J. Burger (Editors)

Associate Editors

John Ashby, William Kelce, Werner Klein, Kenneth Korach,
James Lamb, and Peter Matthiessen



Preferred citation: J. Miyamoto and J. Burger (Eds.)
Implication of Endocrine Active Substances for Humans and Wildlife, SCOPE/IUPAC,
Research Triangle Park, NC (2003). <www.iupac.org/publications/pac/2003/7511/>

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Funded by:

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Chemicals Evaluation and Research Institute, Japan (CERI)
Consortium for Risk Evaluation with Stakeholder Participation
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Pharmacia/Pfizer
Society of Toxicology (SOT)
Syngenta Central Toxicology Laboratory
United Nations Educational, Scientific and Cultural Organization (UNESCO)
U.S. National Institute of Environmental Health Sciences (US NIEHS)
U.S. Environmental Protection Agency (USEPA)

Preface

Disruption of endocrine systems by anthropogenic as well as natural compounds has become an important global issue during the last decade, because it may encompass not only humans, but also a wide range of other organisms, and not only the present generation, but also future ones. Massive scientific research efforts are currently underway to assess the significance of reported adverse effects of exposure to xenobiotics on endocrine systems.

In 1996, the U.S. Congress mandated the EPA to establish the Endocrine Disruptor Screening and Testing Advisory Committee, which issued its final report in October 1998. The Committee recommended testing programs composed of tiered systems, starting with high-throughput pre-screening, followed by tier 1 screening using relatively simple in vivo screening, and tier 2 testing involving laborious and time-consuming tests such as rodent two-generation reproduction studies. Concomitantly, the Organization for Economic Cooperation and Development (OECD) initiated similar programs.

It was clear that there are still many scientific uncertainties to be resolved before acceptable testing procedures can be established. As pointed out in the recent "White Book" on Endocrine Disruptors by IUPAC, International Union of Pharmacology (IUPHAR), and International Union of Toxicology (IUTOX) ["Natural and Anthropogenic Environmental Estrogens: the Scientific Basis for Risk Assessment", *Pure and Applied Chemistry*, **70** (9), 1617–1865 (1998)], these uncertainties can only be resolved by conducting high-quality scientific investigations and a thorough peer review of the results. In this report, the Unions highlighted the need for a better understanding of the mechanisms by which the chemicals produce their effects, and for further examination of the relation between exposure and adverse effects both on humans and the environment. They called for the development of better methods of screening and testing chemicals. Finally, they pointed out the need for a review of existing and new risk assessment methods.

Most of the reviews undertaken about endocrine active substances had considered these issues at a national (e.g., USA, Japan) or European Union level, while the only truly international study, by IUPAC, IUPHAR, and IUTOX, took place at an early stage in the development of research in this field.

The present SCOPE/IUPAC project on endocrine active substance is the only one looking at endocrine active substances (EASs) on a world-wide basis, with emphasis on the specific situation in each region. Thus, a comparative approach was applied to look at the various aspects of the issues from different regional perspectives, and this gives the project significant added value.

Moreover, the project was also designed to take account of the significant advances in the current scientific understanding over recent years. It thus has yielded policy-relevant information and advice that could not be addressed by previous initiatives, due to the early stage of scientific information.

The issue surrounding EASs addressed by the project are a high priority on the decision-making and practitioner agendas regarding environmental chemicals, both in individual countries and globally. Scientific gaps and uncertainties remain high, and will continue for some time. However, the in-depth, comprehensive, authoritative review of EASs and their environmental and health effects by the SCOPE/IUPAC project on endocrine active substances will facilitate risk assessment and assist governmental and intergovernmental authorities, industry, and the wider public, in framing policies to address these issues.

The project culminated in a Symposium held 17–21 November 2002 in Yokohama,

Japan. Scientists, managers, and public policy-makers presented papers in (1) human effects, (2) wildlife effects, (3) exposure assessment, and (4) testing for EASs and endocrine disruption (ED) effects, as well as in six workshops dealing with the effectiveness of QSAR, toxicogenomics, integrated monitoring systems, rapid assays, precautionary principle/weight of evidence approaches, and risk management options for endocrine disruptors. Overall, 408 scientists gathered from 31 countries, giving 84 talks and an additional 84 posters. The papers presented form the basis for a special issue of the journal *Pure and Applied Chemistry*, **75** (11/12), 2003, edited by J. Miyamoto and J. Burger.

J. Burger
SCOPE/Rutgers University, New Jersey, USA

Dedication

On 14 April 2003, Dr. Junshi Miyamoto passed away unexpectedly after a short illness. All the members of the Scientific Advisory Committee of the SCOPE/IUPAC project on endocrine active substances, as well as all the contributors to the project find it hard to accept, given that he was so lively at the Yokohama Symposium in November 2002. With his death, we have lost the spiritus rector of the project and a very good friend.

Dr. Junshi Miyamoto, trained as a chemist, provided impressive scientific achievements during his career in the Japanese crop protection industry, where he had been Associate Director of the board of Sumitomo Chemical for ten years until retirement, and general manager of the Takarazuka Research Center for seven years. His major fields of research were toxicology, metabolism, and evaluation of the mode of action of pesticides and other xenobiotics. He also worked on biochemistry and molecular biology, environmental chemistry, and risk assessment of chemicals. In all, he published over 350 scientific publications.

The major objective of his professional activities was to contribute a science base to issues of safety and public concern and to promote the understanding of chemistry. Consequently, he was active in many international organizations, served IUPAC for more than 25 years in different positions, and was a major contributor to nine Environmental Health Criteria documents of the International Program on Chemical Safety (IPCS). He was also very active in SCOPE and the Intergovernment Forum on Chemical Safety (IFCS). His achievements were acknowledged by many foreign and Japanese awards.

From the beginning of the evolving concerns on endocrine disruption, he engaged himself in the sound scientific judgment of the potential risks for humans and the environment. This involved him in the IUPAC/IUPHAR/IUTOX "White Book" on the basis for scientific risk assessment of natural and anthropogenic estrogens. With continuous public concern and advances in scientific knowledge, and his tremendous efforts, scientific skills, and management capabilities, he initiated and led this current SCOPE/IUPAC project. It is the result of his outstanding leadership that over 80 leading international scientists in this area contributed to this unique and comprehensive project. Thus, it is our honor and deeply felt obligation to dedicate this publication to Dr. Junshi Miyamoto. The members of the Scientific Advisory Committee miss him deeply, as we have all lost a friend and a valued colleague.

Joanna Burger
Co-Chair of the SCOPE/IUPAC Project

Implications of Endocrine Active Substances for Humans and Wildlife: Executive Summary

J. Miyamoto and J. Burger

Introduction

Understanding the scientific issues surrounding endocrine active substances (EASs) is an international priority. The present SCOPE/IUPAC project is a natural extension of the first project by IUPAC/IUTOX/IUPHAR (1998) [1], conducted in 1997. Many recommendations were made at that time, the chief of which was that progress made in understanding and responding to the global problem of EASs and endocrine disruption (ED) should be reviewed as appropriate. As defined during the 1996 Weybridge Workshop, "an endocrine disrupter is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function." Advances in our understanding over the past five years have made it possible to review all of the major aspects of this problem and to refine goals and research needs. The project concentrated on four broad areas: (1) nuclear receptor mechanisms, (2) fate and metabolism of EASs, (3) effects in rodents and humans, and (4) effects in wildlife species. Workshops were also conducted on a variety of technical, regulatory, management, and policy issues.

Each of the four sections included presentations and chapters from experts in the area, and each section resulted in the production of a set of key recommendations. There was a degree of overlap in the recommendations between the sections, with some topics receiving unanimous support from all groups. As might be expected, more detailed laboratory and field experiments and observations are required to move the field forward and to provide sufficient data for all aspects of human and ecological risk assessment of EASs and risk management for human and ecological receptors. In the following summary, the broad sweep of these recommendations is presented. The complete report is available in a special issue of the journal *Pure and Applied Chemistry*, **75** (11/12), 2003, edited by J. Miyamoto and J. Burger [2].

The problem: Scientific knowledge and our current ignorance

The serious and concerted study of ED is barely a decade old, although toxicity tests in some laboratory animals for the types of adverse effects seen with ED date back more than three decades. Despite this, some effects in wildlife were noted much earlier, stimulating great concern. In the last decade, significant advances in our understanding of the underlying biology of endocrine control and the processes of reproduction and sexual development have occurred. Nonetheless, this recent progress has served mainly to highlight our current lack of knowledge regarding not only the underlying biological systems, but also whether low levels of EASs in the environment pose any appreciable degree of risk to humans or to wildlife. The endeavor before us, which commenced as the potential of exposures to relatively high levels of estrogens to induce ED effects in a few species, has grown to include all forms of life on Earth, all natural and anthropogenic sources of exposure to EASs, and a variety of mechanisms of endocrine control. Concomitant with this have been efforts, often uncoordinated, to devise and validate ED assays with which to tackle the task of hazard assessment.

One of the main questions facing scientists and policy-makers is: When is there enough scientific understanding to proceed with actions? We are, therefore, caught between many urgent calls for action, and the realization that the means and knowledge to achieve these actions are only inadequately understood. This has inevitably led to the adoption of simplified models with which to devise assays and hazard definition/risk assessment methodologies. While accepting that this involves necessary compromises, it is important not to forget that these compromises have been made, and to remain open to the impacts that new insights and understanding will have on these simplified, yet enabling models. For example, it is known that at the molecular level, the processes that regulate cell growth, differentiation, and/or function can be ligand-dependent or -independent, and that the consequent biological effects can be induced through both genomic and nongenomic regulatory pathways. Nonetheless, this complexity has to be expediently reduced to the enabling model in which xenobiotic ligands bind to nuclear receptors, leading to the induction or inhibition of downstream gene expression. The value of such simplified models applies to all aspects of this endeavor, and they remain valuable only so long as they are not allowed to outlive their usefulness.

There is a need for an international forum capable of assessing the impact of major advances in understanding, or of methodological advances, and relating these to current testing and risk assessment strategies. While the Organization for Economic and Cooperative Development (OECD) has contributed admirably in the test methods validation efforts, the pace has been slow. This international effort should involve basic and applied researchers from academic and research institutions, government agencies and laboratories, scientists, industrial and contract research facilities, and government regulators. Such a forum should consider new data on target tissues, critical life phases of exposure, refined endpoints, dose response, and improved methods of data interpretation. At present, this is done only at the national level, and often less than comprehensively.

In the following sections, human effects, wildlife effects, exposure, testing for EASs, and the importance of assays conducted in vivo are discussed. Each section contains an overview, recommendations, and management considerations. These sections are followed by a section of generic issues germane to the entire field of EASs and ED.

Human effects

It is important to confirm the postulated range of human effects and their possible reversibility currently ascribed to EASs. It is necessary to establish whether exposures to EASs are causative, contributing, or unrelated to effects. Some early reports, for example, a decline in sperm counts, have not been confirmed in all locations by subsequent more extensive investigations. Other reported effects include acceleration or delay in the timing of human sexual maturation and increases in the incidence of human sexual developmental disorders. The role of EASs in these observations remains to be determined. Nevertheless, it is important to continue to research what role, if any, exposures to EASs play in established human epidemiologic trends, such as increases in incidence of cancer in hormone-sensitive tissues (e.g., testis, breast, prostate). The potential for effects occurring in utero following maternal exposure to EASs also requires further study. Some diseases may be evident only after chronic exposure or after a long latency. Therefore, even though toxicity testing methods, such as the developmental toxicity study and the mammalian multigeneration reproduction study, are capable of detecting ED disease conditions, including those unique effects that may occur from exposures in utero, as our knowledge increases, there is a need to consider revising such methods and perhaps developing newer, more sensitive and specific techniques. It is too early to reach firm conclusions about whether human populations are seriously at risk from potential exposures to EASs, and further vigilance is clearly required. However, it is somewhat reassuring that after substantial research in the past decade, there have been no conclusive findings of low-level environmental exposures to EASs causing human disease.

Chemical interferences with steroid biosynthesis and metabolism can produce adverse health effects, even though the inducing agent would not be detected as an EAS using receptor-based test systems. This is an important area of study because some examples of ED occurring in animals derive from exposure to inhibitors of steroidogenic enzymes such as 5α-reductase and aromatase. Some such agents are known to be active in humans and are used successfully in the treatment of a range of human hormonal conditions. Evaluation of such effects requires integrated screening that brings together in silico, in vitro, and in vivo technologies.

The following are research priorities and recommendations:

- Focus epidemiologic studies on testing hypotheses to understand risk factors, including exposure to low levels of EASs on acceleration or delay in puberty and its consequences to health and well-being.
- Study beneficial and hazardous effects of phytohormone and synthetic hormone exposure, including relative dose and exposure levels.
- Evaluate dose response and consequences of maternal exposure to EASs on development of offspring, including elucidation of mechanisms of effects.
- Identify sensitive developmental windows of exposure to natural and synthetic EASs and describe dose– and time–response relationships to advance our understanding of the potential for long-term effects that may arise well after exposure.
- Examine putative atypical dose-response curves with regard to overlapping mechanisms of action.
- Conduct experimental studies on the impact of mixtures, as well as the interaction of xenobiotics with endogenous hormones.
- Conduct epidemiologic studies of the consequences of exposures to defined EAS mixtures.
- Address the need for epidemiologic studies of putative endocrine effects that carefully take into account potential confounders and alternative risk factors.
- Use molecular epidemiology approaches to identify susceptible populations.

Wildlife effects

Field studies have shown that many individual organisms and populations have experienced some degree of exposure to EASs, and in some cases, both individuals and populations have been adversely affected. In some cases, these effects have influenced population stability and/or the integrity of relevant animal communities. Over 200 species are either known or are suspected to have been affected by EASs, including examples from at least two invertebrate phyla and all five major vertebrate classes. Although scientific knowledge of EAS interactions with wildlife species is accumulating, we are still struggling to answer the question of whether low levels of exposures to environmentally relevant concentrations of EAS in the environment pose an appreciable risk to many species. Field observations indicate there are sometimes large differences between closely related species and between individuals of the same species, but the factors that are responsible for these differences, such as differential exposures or differential metabolism, are poorly understood.

Most examples of ED in wildlife have been reported from Europe, North America, Japan, and Australasia, but this may simply reflect the current global distribution of research effort in ED. In general, the effects reported are confined to the more contaminated areas of the planet, although studies have shown that lower levels of exposures to certain substances can occur in areas far from the vicinity of large human populations (for example, exposures have been documented in the Arctic—an area previously considered pristine), presumably caused by the atmospheric distribution of persistent and bioaccumulative EASs. Most examples of ED in wildlife are associated with aquatic species and with consumers of aquatic species; these examples have been attributed to high or continuous exposure to EASs experienced by water-breathers and their predators. However, this conclusion may have been influenced by the fact that the majority of wildlife ED research has been focused on aquatic life. The large majority of current investigations have concentrated on the individual, but of greater potential concern may be effects on the population. Population effects, however, are difficult to discern without long-term population data sets.

The EASs associated with impacts on wildlife include representatives of natural and synthetic steroids; synthetic alkylphenols; natural phytoestrogens and phenolics; natural and synthetic polycyclic aromatic hydrocarbons (PAHs); and synthetic organohalogens and triorganotins. Some of these substances are now subject to government regulation, and for some of these substances, regulatory action has been accelerated because of ED concerns. Other actions are expected as the newest scientific knowledge of exposures and hazards is evaluated in the near term by regulatory agencies. However, those EASs that we currently recognize probably form only a small proportion of the total EAS burden in the environment. Perhaps the most intractable of current problems is that posed by natural steroids excreted by humans and livestock and discharged in a reactivated form in sewage effluents and in runoff from agricultural operations. In these cases, upgrading of sewage treatment plants is the appropriate solution.

The major recommendations for future wildlife studies are as follows:

- Vertebrates (terrestrial and aquatic) possess similar endocrine systems and can act to some extent as surrogates for each other in testing or monitoring programs. However, the >30 invertebrate phyla (comprising about 95 % of all known animal species) have diverse and often poorly understood endocrine systems. Apart from the long-term need to study these different systems, there is a current need to define sentinel species to reduce the impossible workload

of studying all species to the same degree. Although it is probable that a relatively small number of screening tests will be sufficient to identify the large majority of potential EASs, a full range of apical (comprehensive, multi-endpoint) assays such as the rodent multigeneration assay and the fish life cycle test, will be required to clarify the environmental hazards posed by EASs to at least six invertebrate phyla and all classes of vertebrates. These apical assays must be practical and properly validated, and should be capable of measuring mixture and low-dose effects and anticipating population effects.

- The potential differential sensitivity to EASs of different life history stages and reproductive strategies in the many species subject to ED requires study. For example, in certain fish, processes such as hermaphroditism, reproductive behavior, larval-to-adult metamorphosis, smoltification, and osmoregulation are under endocrine control, but the response of these to EASs has yet to be systematically considered.
- There is evidence from studies conducted in fish that chronic exposure to a wide range of contaminants can inhibit normal responses to stress. The wide implications of this finding require further study. There is also surprisingly limited information on the existence of xenobiotics that mimic or antagonize the activity of corticosteroid hormones or interfere with the catecholaminergic system.
- Consideration should be given to potential interactions between the estrogen/androgen systems and the immune, corticosteroid, catecholamine, thyroid, and retinoid systems. At present, such interactions are either not considered or are not adequately illuminated due to experimental use of only a limited range of reference substances and because dose–response studies are often not integrated into study designs.
- It is critical to identify factors that could confound interpretation of suspected ED effects in wildlife. This will involve collection of appropriate background data from relatively uncontaminated environments and appreciation of natural variability. The factors that could cause natural variability include changes in the levels of steroid binding proteins, and differences in intrauterine positions, temperature, and food/water availability.
- There is a need for laboratory and field studies to separate the effects of different EASs that may be acting together, leading to greater understanding of the impact that mixtures have on wild populations.
- At present, only a small fraction of the many wildlife species and different ecosystems have been investigated with respect to ED. This increases the need to assess the causes and risks of ED in endangered species, many of which are found in tropical regions that so far have not been studied in this respect. This will require the development of noninvasive monitoring techniques, such as nondestructive biopsy.
- There is a need for development of robust population and ecosystem models that can be used to predict higher-order effects from knowledge of responses observed in individuals. Validation of these models is important.

Exposure assessment

Understanding and quantifying exposure is an essential feature of risk assessment and management. Exposure assessment includes the sources, fate, and transport of EASs in environmental media, contact with organisms, bioavailability and absorption, and distribution to target tissues or receptors. There is a substantial existing literature on exposure assessment to chemicals in general, which should be applied to EASs.

Monitoring programs for EAS occurrence and effects are important. Systematic sam-

pling of indicator species and measurement of biomarkers should inform environmental management and provide early warning of problems. In coordination with these biological studies, reliable and valid information on the fate and transport of substances in the environment and humans is a prerequisite for the design of monitoring programs. Monitoring should identify and document changes in the spatial or temporal occurrence of hazards or risks from environmental media and foods. Indicators should include sensitive species or population subgroups. Sustained monitoring programs should include high-priority EASs, which may pose a risk for humans or the environment. Such programs require sustained investment and public support.

Retrospective studies using appropriately archived environmental and human samples can provide valuable baseline data, and appropriate archiving of biological and environmental samples for future studies is essential. This will allow further and future analysis for substances not recognized at the present or the use of more sensitive analytical technologies.

- Existing monitoring programs (e.g., the U.S. EPA's EMAP program) should be expanded to include EASs, and data should be compared to models to enhance exposure assessment and achieve comprehensive risk assessment for EASs.
- Such monitoring programs should be implemented in countries with economies in transition.
- Identification of the relative contribution of different EAS components of an environmental medium can be accomplished by toxicologically guided fractionation and analysis of complex environmental media, known as toxicity identification and evaluation (TIE). This approach can help set priorities for management.

Measurements of contaminants in the environment are important, but do not necessarily reflect internal exposures or dose of EASs to target organs. Dose to target is influenced by variation in bioavailability and absorption, and metabolism and transport. Care is, therefore, necessary when relating environmental measurements to predicted exposure or effects.

Specific research priorities to improve exposure assessment include:

- There is a need to conduct more field monitoring of exposures to highly potent EASs.
- Research is needed to investigate chemical activation (e.g., by hydroxylation of non-active substances), which may influence exposure to or uptake of EASs.
- Research is needed on hormonally active pharmaceuticals and dietary supplements, particularly those or their metabolic products that are excreted into sewage. Such studies should include reactivation of conjugated metabolites in the environment.
- Improved exposure modeling and parameterization require more extensive data from field studies. This includes data on individuals and sensitive life stages, and seasonal or other variables. Field validation of models is particularly necessary.
- Processing of plant materials and consequent recycling and redistribution of phytohormones into the environment requires careful observation and monitoring. Examples include paper-pulp mills, food processing, and sewage effluents.
- Maternal–fetal exposure from dietary intake of phytohormones warrants additional research and monitoring.
- Chemical analytical methods have been developed, providing adequate detection limits and precision for the analysis of the most important groups of known EASs in food and the environment at levels of biological significance. Technical improvements are needed to simplify sample preparation, diminish confounders, enhance analytical sensitivity, and reduce cost.

Testing for EASs and ED effects

There is a growing range of assays suitable for defining the potential of chemicals to interact with several hormone receptors in vitro, perhaps also with concomitant expression of hormone receptor-regulated genes, or with steroidogenic enzymes in vitro. However, these assays cannot define ED activity, as there is no endocrine system being monitored. To gain information regarding whether an EAS will show ED activities, it is necessary to use assays based in a whole organism—such as the fish vitellogenin assay or the rodent uterotrophic and Hershberger assays. Above this class of assay are the apical test systems, such as the rodent multigeneration assay and the fish life cycle assay. The primary distinctions between the different classes of assay will be important to recognize when devising any form of coherent or tiered testing strategy.

The following points were considered important for future development of more efficient testing schemes:

- Individual (mechanism-dependent) structure–activity relationships (SARs) derived in silico, may be of value in prioritizing the evaluation of functional and structural congeneric classes of chemicals. However, SARs may be of limited value when screening all classes of chemicals for all possible mechanisms of action and for interactions with numerous molecular components of the endocrine system.
- Attempts should continue to enhance higher-level testing by adding more sensitive endpoints and maximizing use of the animals being studied. However, care must be taken not to overburden experimental protocols leading to reduced overall efficiency.
- Agreement should be sought on a base set of comprehensive test systems that will be capable of confirming or over-ruling initial indications of endocrine activity provided by lower-tier tests.
- There is a need to improve dose–response analyses and to understand how individual system characteristics can create different dose–response relationships. This is important for moving risk assessments away from default assumptions to more scientifically based approaches.
- Studies in amphibians and humans have established the developmental importance of the thyroid gland, and inclusion of evaluations of TSH, T3, and T4 assays in multigeneration, developmental, and neurotoxicity test protocols has recently been considered. Further investigations are required to establish the optimum way to monitor dose- and time-dependent changes in thyroid function in relation to exposures to postulated EDs and EASs.
- Further work is required regarding extrapolation of benchmark doses, derived from experimental animal studies, to predicted safe human or ecological exposure levels.
- Chemical analytical methods exist with adequate detection limits and precision for the analysis of the most important groups of known EASs in food and the environment. Refinement of these methods could include provision of more specific and sensitive biomarkers, better integration for diverse classes of EASs, and more automated and selective clean-up procedures. Increased use of LCMS and LCMSMS and stable ELISA tests could contribute to improved quality assurance.

Importance of assays conducted in vivo

Although discrete mechanisms of ED action can be studied using assays in vitro or in silico, interactions within the endocrine system cannot be assessed in a comprehensive way by simple in vitro assays. Consequently, data from in vitro assays that assess specific aspects of ED activity should be combined with more apical short-term in vivo screens to capture substances interacting with the endocrine system at more complex levels. Such in vivo assays are uniquely able to take account of factors such as the following:

- Metabolic activation and/or deactivation of the EAS, including induction by nuclear receptors (pregnane x receptor and/or constitutively activated receptor) to increase cytochrome-P450 enzyme activity (Phase I metabolism), increase conjugating enzyme activity (Phase II metabolism), or increase drug transport proteins (*p*-glycoprotein), all of which affect exposure.
- Integration of the biological half-life of EASs and their relative binding affinities to chaperone proteins and receptors, including coactivator/corepressor interactions and growth factor interactions and signaling cross-talk.
- Cell- and tissue-specific expression of nuclear hormone receptors and their isoforms and phosphorylation states, including compensation through functional redundancy (receptor isoforms and/or convergent signaling pathways) and repair processes.

Overarching issues in EAS research

A range of needs were identified that apply to all aspects of the study of ED and EASs, as follows:

- The field of ED is rich in unexpected observations—consistent with evolving methodologies and the state of our understanding of the underlying biology of the endocrine systems. However, the science will be aided by a renewed commitment of researchers to follow the scientific method, by testing hypotheses, confirming unexpected findings (wherever possible, before publication), communicating data and results clearly, and including in analyses several alternative, biologically plausible and reasonable explanations for observations.
- Uncertainty regarding whether some EASs may possess the ability to induce effects at doses below those considered safe using current testing methodologies should be evaluated urgently and resolved. Central to this will be agreement on an EAS in a named system that shows such a nonmonotonic dose response. This will enable progress on establishing the range of dose–response relationships that may exist for EASs (monotonic/nonmonotonic, threshold/nonthreshold). It will also enable the molecular mechanism of nonmonotonic responses to be studied.
- Pharmacodynamic and pharmacokinetic factors, and the half-life and bioaccumulation potential of chemicals, should be incorporated into all risk assessment strategies. This requirement means that the route and method of chemical administration adopted in animal tests should be considered and justified, because such decisions may have a profound influence on the quality of the data generated and on their ability to be extrapolated to humans and wildlife species.
- In all areas of study, there may be sensitive subgroups of exposed individuals that may show much greater responses than

the majority. This could lead to a loss of information if this potential is not recognized. We need better statistical methods to detect such effects, which may be obscured by population-based, parametric statistical analysis alone.

- Research should clearly identify statistical issues related to underlying variability in exposure or response, sample size, and power. Graphical representations should clarify this variability and identify individual outliers with unusual exposure or responses, which may be of biological or ecological importance.
- Field studies are expensive to mount. Therefore, in all such cases, the possibility of cryo-preserved archiving of samples should be considered pending advances in assay techniques.
- Methodologies need to be developed to improve quantitation and understanding of both the certainties and the uncertainties associated with extrapolating experimental animal data, derived from multiple studies using different endpoints, to effects expected at ambient levels of environmental exposure.
- Consensus on definitions and applications of the *precautionary principle* and the *weight of evidence approach* should be sought. At present, these two concepts compete for attention and are subject to a range of definitions. For example, some regard the former as taking action in the absence of complete information in case a hazard should exist, but others regard it as taking action when an overwhelming case has been made, but which falls short of absolute proof. The precautionary approach also allows action when the probability of an adverse effect may be low, but consequences are considered large and/or irreversible, and the cost of preventive action is acceptable to society. Likewise, some regard the weight of evidence approach as a numerical averaging of positive and negative data sets, while others regard it as an expert integration of all available data, that may include the explicit consideration of some clear, but isolated positive or negative findings. The integration step includes consideration of the adequacy, strength, and consistency of the overall data set as well as the coherence of results with respect to toxicological relationships between affected endpoints and commonality of underlying mechanisms. Because screening assays provide qualitatively different information than definitive tests, the results from these dissimilar assays are used in a manner that is consistent with the scientific basis and purpose of each. To advance our understanding of the relative merits and disadvantages of these different approaches to risk management, it is essential to examine some examples of actions that have been taken on EASs, compare the different outcomes, and decide which are preferable.

Conclusions

That natural substances as well as synthetic chemicals have the potential to interact with the endocrine system of organisms in complex and often subtle ways is no longer surprising. It is well established that certain substances can interact with components of the endocrine system and produce adverse health effects. One important question is whether low levels of exposure pose any appreciable risk. Although low levels of some EASs are already known to have caused adverse effects in some wildlife species, the reported pervasiveness of effects attributable to low doses of some synthetic EASs invites validation. Failures in the past to provide full and complete data sets for scientific expert panel analysis cannot be perpetuated, because such actions impact the integrity and credibility of the research. Furthermore, the risk management implica-

tions of low-dose effects are potentially substantial and warrant careful scientific replication. Although the human and ecological consequences of ED may not be as universal as some have feared, there are sufficient examples and biological plausibility to leave little basis for complacency in the research community. Future well-designed research, encompassing temporal, spatial, and taxonomic trends, exploring multiple mechanisms of action, and clarifying interactions between endocrine and other (nervous, immune) systems, will elucidate the magnitude of the problem, identify target substances of concern, and advance our knowledge of human and wildlife health. In cases where there is documented scientific evidence based upon valid studies of serious and irreversible damage, but some degree of scientific doubt, it may be important to consider implementing interim precautionary measures or risk management actions that may avert harm, while ongoing research fills the knowledge gap. Risk assessment techniques that apply additional safety factors to make up for the lack of information and uncertainty of the quality of the database or suspected greater sensitivity of the subpopulation is an example of such a precautionary approach. Where biological systems appear well adapted to perturbations, a graded intervention may be acceptable. To tackle these problems, the Intergovernment Forum on Chemical Safety (IFCS) should, therefore, initiate integrated global management of the ED issue. At present, there is little, if any, coordination between research findings and national and international societal responses. Such cooperation and coordination is essential to further research and wise management of the EAS issue.

In conclusion, we have learned that the global effects attributed to EASs are not as all-pervading or fearsome as some have asserted, nor as trivial as others would wish. The beauty of science is that "more research is always needed", and our quest for understanding the world around us is boundless. However, the most important question regarding ED is: What are the significant effects of EASs in terms of health, well-being, and population stability of humans and wildlife?

References

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