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Risk perception: A chemical industry view of endocrine disruption in wildlife*

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Abstract: Manufactured chemicals are essential to the vast array of goods and services that contribute to modern life. Their benefits are innumerable, and society is entirely dependent upon them. At the same time, there is an increasing awareness of the concept of environmental impacts. The challenge is to achieve the appropriate balance between the benefits and risks from chemicals, so that we all may enjoy the benefits of chemicals without significant detriment to current and future human and wildlife health. Ecological risk assessment is the mechanism that allows potential environmental chemical exposure to be benchmarked against hazardous properties so that risk is acceptable and environmental health is not impaired. Chemical management decisions based on such assessments are said to be risk-based. Within the context of environmental risk assessment practice for endocrine disruption, industry would support a position that:

- endorses the risk assessment process;
- recognizes that endocrine disruption is not an adverse effect per se, but rather a potential mechanism of action;
- gives precedent to population-level effects instead of individual-level effects;
- employs a tiered approach to hazard assessment;
- emphasizes, standardizes, and validates effects testing methodologies;
- recognizes that exposure per se does not necessarily constitute a risk;
- considers relative potency (i.e., evaluation of the dose levels and mechanisms producing toxic adverse effects and determining whether the critical effect arises via an endocrine mechanism or another mechanism);
- benchmarks risk against loss of benefits; and
- evaluates risk within the context of overall risk from both natural and anthropogenic substances with common modes of action.

To help address uncertainty surrounding the risk from endocrine active substances (EASs) to wildlife, the chemical industry—via the Long-Range Research Initiative (LRI)—has implemented a research program aimed at identifying and addressing knowledge gaps and establishing internationally harmonized testing methodologies in cooperation with other stakeholders. Details of individual projects within the current LRI research program are presented.

*Report from a SCOPE/IUPAC project: Implication of Endocrine Active Substances for Human and Wildlife (J. Miyamoto and J. Burger, editors). Other reports are published in this issue, *Pure Appl. Chem.* **75**, 1617–2615 (2003).

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INTRODUCTION

Manufactured chemicals are essential to the vast array of goods and services that contribute to modern life. Their benefits are innumerable, and society is entirely dependent upon them. At the same time, there is an increasing awareness of the potential environmental impacts of chemicals. Consequently, there are increasing stakeholder demands for public information about chemicals in consumer products and about emissions to the environment. The implicit aim of these stakeholders is one of encouraging less hazardous production and reduced use of chemicals in general. The challenge to civil society is to achieve the appropriate balance between the benefits and risks from chemicals, so that we all may enjoy the benefits of chemicals without significant detriment to current and future human and wildlife health. However, it is inevitable—given the variety in volume and scope of physicochemical characteristics of chemicals in commerce—that some environmental exposure of both human populations and wildlife in the ecosystem will take place. However, exposure per se does not constitute risk. Likewise, a compound may be potentially hazardous based on intrinsic properties, but only via exposure will it be able to exert such potential and result in adverse effects. It will not pose a risk where exposure is limited. This was recognized by Paracelsus in his oft-quoted dictum "*Sola dosis facit venenum—the dose makes the poison*". Risk is therefore a function of both exposure and potential effects.

Ecological risk assessment is the mechanism that allows potential environmental chemical exposure to be benchmarked against hazardous properties so that risk is acceptable and environmental health is not impaired or otherwise impacted. Chemical management decisions based on such assessments are said to be risk-based. Risk-based approaches differ from exposure- and hazard-based approaches. Exposure-based decisions relate to pollution prevention or minimization of exposure irrespective of chemical properties. Hazard-based decisions employ risk avoidance by avoiding any exposure to chemicals with specific hazards (e.g., carcinogens, teratogens). When decisions are hazard- rather than risk-based, the implication is that any exposure to a chemical with the specific inherent properties under consideration is unacceptable, regardless of actual risk.

With the advances of risk and exposure sciences during the last 20 years, decisions to control, limit, or otherwise manage the use of chemicals are increasingly risk-based. Risks can and should be scientifically assessed using actual data when available. To garner the confidence and acceptance of the scientific community, public, and stakeholders, risk assessments should be conducted in a scientifically rigorous manner, using the best available/scientifically credible hazard and exposure information, and risk assessment techniques and reasonable safety factors. All relevant information should be made public. The resulting risk assessment should be scientifically defensible and therefore open to review by independent scientific experts. These basic principles are included in the International Council of Chemical Associations' (ICCA's) "Principles for Risk-Based Decision Making", which describes a reasoned approach to protecting the environment and public health [1].

During the last decade, considerable research has focused on evaluating potential hazards, exposures and impacts from exposures to environmentally relevant levels of EASs on human health and ecological well-being. The "endocrine disruptor hypothesis"* postulated that certain substances could act like natural hormones and, even at low levels typically found in the environment, could cause adverse effects by interfering with the endocrine systems of wildlife and humans. To help address uncertainties, it was recognized that considerable research would be necessary to increase scientific understanding and determine whether adverse effects in humans and wildlife were arising from hormone-mediated

*As defined during the 1996 Weybridge Workshop [5], "An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function." It is generally understood that the definition of "endocrine disruption" encompasses both the endocrine mechanism of action and adverse health effects. Such a definition is consistent with our understanding of the science in that it implicitly recognizes that while substances may interact with the endocrine system, they may not adversely affect health or the ecosystem. For example, natural variations in hormone levels and reversible or transient effects associated with many natural or synthetic substances are well known.

processes from exposure to chemicals in the environment. The chemical industry—via the LRI—implemented a research program aimed at identifying and addressing knowledge gaps and establishing internationally harmonized testing methodologies in cooperation with other stakeholders. Working with academic and research institutions and governments, members of the chemical industry in Europe, North America, and Asia under the aegis of the ICCA sponsor fundamental research on the potential of chemicals to interact with and affect the hormone system and cause adverse effects. ICCA members have pledged to conduct research through an open and transparent process at institutions selected through a competitive peer-reviewed process. The results of this research will be made available to the public and acted upon by industry in a timely manner. An overview of individual projects within the current LRI research program is presented below within the context of specific research recommendations from the Scientific Committee for Toxicity, Ecotoxicity, and the Environment (CSTEE) [2] and International Program on Chemical Safety (IPCS) [3]. Full details of individual research projects can be obtained from the LRI Web sites (<www.cefic.org/lri>, <www.endocrineresearch.com>, and <http://www.nikkakyo.org>).

ENDOCRINE DISRUPTION IN WILDLIFE

IPCS [3] evaluates the extent to which wildlife—both vertebrate and invertebrate—have plausibly been affected by environmental exposures to EASs. Case studies involving mammals, birds, reptiles, amphibians, and invertebrates were all considered (see Table 1). That there are examples of effects observed in wildlife associated with EASs is apparent, particularly in areas "which have received extensive chemical contamination". Many of the case studies have previously been highlighted in other reviews such as Van Der Kraak [4] and CSTEE [2]. The case studies also demonstrate the problems regarding the establishment of cause and effect relationships between chemical exposures and physiological dysfunction across the various taxa considered—particularly when compounded by a wide variety of other potential influential factors such as habitat, food availability, etc.

Table 1 Wildlife case studies from the IPCS-GAED report (from IPCS [3]).

Case studies and associated contaminants	Conclusions
Mammals:	
• Reproductive dysfunction in mustelids (PCBs, dioxins, furans, OC insecticides)	• *"Sufficient evidence that feral mammals have been adversely impacted by environmental contaminants."*
• Reproductive dysfunction in marine mammals (PCBs, DDE/DDT)	• *"Limited evidence….that these effects are mediated through endocrine dependent mechanisms."*
• Reproductive dysfunction in feral rodents (PCBs, cadmium)	• *"Difficult to assess the mode of action of environmental chemicals in feral mammals" due to a "lack of knowledge regarding their endocrinology & reproductive biology and how other environmental stressors affect these processes."*
• Defects in reproductive, endocrine, and immune systems of Florida panthers	
• Pseudohermaphroditism in bears (teratogenic herbicides, androgenic plant alkaloids, PCBs)	
• Pathological lesions in marine mammals (PCBs, DDT/DDE/DDD, PHAHs)	

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Table 1 (*Continued*).

Case studies and associated contaminants	Conclusions
Birds: • Alterations in behavior/reproductive success of colonial waterbirds (DDE, PCBs, mirex/photomirex) • Abnormal reproductive morphology (dioxins, PCBs, mirex) • Sex ratio skew/female–female pairings in gulls (DDT) • Eggshell thinning (DDT/DDE) • Great Lakes Embryo Mortality, Edema and Deformity Syndrome – GLEMEDS (PCHs, PCBs, DDT, dioxins/furans)	• *"Oviparous reproductive strategy and certain life history traits create avenues of exposure that makes these species more vulnerable to EDCs than traditional animal models or humans."* • *"Although exposure to environmental contaminants can have dramatic effects on endocrine regulated process and overall population fitness, the mechanism need not involve endocrine disruption."* • *"Individuals may experience endocrine disruption, which may or may not be linked to effects on reproduction and population fitness."*
Reptiles: • Developmental abnormalities in Lake Apopka alligators (dicofol, DDD, DDE, chloro-DDT, PCBs, dieldrin, endrin, mirex, oxychlorodane) • Developmental abnormalities in Great Lakes snapping turtles (PCBs, dioxins/furans)	• *"Some developmental processes in reptiles…are susceptible to endocrine disruption."* • *"Although some reptile populations have been impacted by environmental contaminants with endocrine-disrupting properties, it is unclear how widespread the phenomenon is."* • *"There is insufficient data to evaluate whether aquatic reptiles are at greater risk of endocrine disruption compared to terrestrial reptile species for which we have limited data."*
Amphibians: • Amphibian population changes (DDT, DDE, DDT, dieldrin) • Deformities/malformations ("retinoid mimics," PCB 126)	• *"There are insufficient data to implicate EDCs as causative agents in amphibian declines."* • *"There is not enough conclusive evidence to state that environmental contaminants are responsible for the observed malformations."*
Fish: • Vitellogenin induction in juvenile male fish/altered gonadal development (estrogenic compounds in sewage effluent, 17β estradiol, estrone, 17α ethinyl estradiol, alkylphenols, alkylphenol ethoxylates, phytoestrogens in pulp/paper-mill effluents) • Reproductive abnormalities/altered sex steroid levels induced by pulp/paper-mill effluents (β sitosterol, sterols, lignans, stilbenes, resin acids) • Reduced reproductive success and population-level effects (PAH, DDT/DDE, PCBs, dioxins) • Altered adrenal physiology (PAHs, PCBs Scientific Committee for Toxicity, Ecotoxicity and the Environment, heavy metals) • Early life stage mortality (dioxins, furans, PCBs) • Thyroid dysfunction (PCBs, perchlordecone, mercury petroleum hydrocarbons)	• *"Endocrine disruption is undoubtedly occurring in wild fish populations."* • *"In most cases the precise modes of action are poorly understood."* • *"The compounds responsible for the observed effects may be due to both synthetic and natural compounds."* • *"There is limited understanding of how the existing endocrine disruption affects population fitness."*

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Table 1 (*Continued*).

Case studies and associated contaminants	Conclusions
Invertebrates: • Imposex in gastropods (TBT) • Disruption of ecdysteroid-regulated processes in crustaceans (insecticides) • Disruption of juvenoid-regulated processes in crustaceans (methoprene, atrazine, nonylphenol) • Molting disturbances and deformities in insects (fenoxycarb, tebufenozide, phytoecosteroids, " DDE, heavy metals, pulp/paper-mill effluents)	• *"The diversity of the invertebrate phyla creates numerous challenges in determining the potential risks of EDCs" to invertebrate health.* • *"Compounding these challenges is the poor understanding of the endocrinology of most invertebrates.* • *"The effects of EDCs in vertebrates will not necessarily be similar to those observed in invertebrates."* • *"Conversely invertebrates are susceptible to endocrine-disrupting properties of compounds that are not problematic in vertebrates."*

EPISTEMOLOGICAL FRAMEWORKS—ASSOCIATION AND CAUSALITY

An endocrine disruptor is defined within the context of an adverse effect upon a complete or intact organism [5]. In contrast, the goal of ecological risk assessment is to protect communities and the functioning of ecosystems from the effects of chemical pollutants. Historically, it has proved problematic to categorically demonstrate that adverse effects at the ecosystem or population level have resulted from exposure to an EAS. Van Der Kraak [4] recognized that defining the extent to which environmental chemicals affect the functioning of the endocrine system and consequently contribute to adverse health effects in wildlife is a "controversial and highly charged issue." This was attributed to a lack of defined criteria for establishing causality vis-à-vis chemical exposure and any observed adverse effects. Identifying a possible association between chemical exposure and an observed adverse effect typically involves an element of speculation. The presence of a compound with a known mode of action in the environment offers the possibility that such exposure is responsible for the observed effect. Such associations of an observed effect with a possible cause are relatively easy to identify and examples abound. Demonstrating that an actual causal relationship exists is much more difficult. Various guidelines and criteria have been applied to aid assessments of causality [3,4,6]. The application of such frameworks allows expert judgement as to whether an association is causal or not. These are qualitative assessments and not quantitative risk assessments that ascribe probability of adverse effects. They can also be used for gap analysis to guide future research needs for individual cases. The starting point is a hypothesis linking an adverse effect to a particular stressor via an endocrine-mediated mode of action. Evaluation is typically on the basis of the five aspects listed below (Table 2), with an overall evaluation of the strength of evidence regarding the linkage between the adverse effect and exposure.

Examples of the use of the framework relating to wildlife effects as dealt with in the IPCS-GAED report [3] are summarized in Table 3. Strong evidence for an EAS-mediated adverse effect was noted for TBT-induced imposex in gastropods, vitellogenin induction in male fish exposed to sewage effluent, and reproductive alterations in fish exposed to bleached kraft-mill effluent (BKME). Moderate evidence for an EAS-mediated adverse effect was noted for decreased reproductive function in Baltic seals, eggshell thinning in birds, and reproductive abnormalities in Lake Apopka alligators. Overall, the evidence that wildlife has been impacted adversely following exposure to EASs is deemed "extensive" —particularly in areas where levels of environmental contamination are known to be high. This is despite the problems inherent in determining the extent of potential effects of EASs on wildlife (i.e., exposures to multiple substances and a high number of potential species with differing life histories, physiology, and endocrine systems). The key unresolved question identified in the report is whether areas

with lower (background) levels of contamination likewise constitute a risk to wildlife. The examples cited relate to aquatic ecosystems that are highlighted as sinks of environmental chemicals, particularly those that bioaccumulate (i.e., PCBs, dioxins/furans, and DDT/DDE). Many of the compounds highlighted have essentially already been eliminated from production (e.g., PCBs, DDT) or otherwise restricted (e.g., TBT), although some remain widely distributed in the environment.

Table 2 Framework for evaluation of causality (from IPCS [3]).

Element for evaluation	Aspect
Temporality	Does exposure precede adverse impacts upon health?
Strength of the association	Examination of the incidence rate in a population, the extent to which other factors may have contributed, the risk attributable to exposure, and shape of the dose–response curve.
Consistency of the observations	How frequently are similar or dissimilar observations made (across regions, across species, across exposure)?
Biological plausibility	Evaluation of the mechanism of action of the compounds of concern. Is the substance an endocrine disruptor?
Evidence of recovery	Examines whether the adverse effects are reversible if exposure is reduced/removed.

CENTRAL TENETS FOR RISK ASSESSMENT OF ENDOCRINE ACTIVE SUBSTANCES

The examples of effects of EASs on wildlife highlighted above amply illustrate the need to establish appropriate tools with which to assess risk in both a prospective fashion for new chemical entities and a retrospective fashion for existing compounds in commerce. In proposing the use of risk assessment in this context, there are several central tenets or guiding principles to promote a scientifically rigorous approach for assessing and managing potential risk.

Risk assessment concept

Hutchinson et al. [7] detail a tiered approach to ecological risk assessment for suspected EASs. This framework draws from the previous suggestions by Stevens et al. [8,9] that, without evidence to the contrary, it is scientifically sound to assess and evaluate endocrine-related effects in the same manner as adverse effects caused by other modes of action. This stance was reiterated by the CSTEE [2] who confirmed that the underlying concept of risk assessment is scientifically sound and applicable to the issue of endocrine disruption. Due to regulatory considerations, this focus on ecosystem structure and function is modified somewhat and ecotoxicological assessments are based on a limited number of laboratory species as a consequence of technical and economic considerations. However, this concept is still appropriate if the ecotoxicity assessment is conducted with representative taxonomic groups, ecologically relevant endpoints (i.e., reproduction), and acceptable extrapolation from laboratory to ecosystem. The concept of ecological risk assessment is therefore general and not related to mode of action [2]. The ecotoxicological consequences of endocrine disruptors should therefore be assessed by general nonspecific endpoints relevant for the detection of population–community effects, but the tests must cover potential consequences of hormonal alterations. The approach is questionable only if ecotoxicity testing is unable to detect toxicity/endpoints related to endocrine disruption or when the uncertainty factors/probability cut-offs employed in extrapolation from the laboratory to the ecosystem are not appropriate (i.e., acute/chronic, chronic/multispecies ratios).

Table 3 Illustrative examples of the evaluation of the association between environmental exposure to EASs and adverse wildlife effects (from IPCS [3]). Each element was ranked from weak (*) to strong (****), and the overall strength to support the hypothesis and EAS mechanism was evaluated as either weak, moderate, or strong.

Statement of hypothesis		Evaluation factor					Overall strength of evidence	
Outcome	Stressor	Temporality	Strength of association	Consistency	Biological plausibility	Recovery	For hypothesis	For EAS mechanism
Imposex in marine gastropods	Tributyltin (TBT)	****	****	****	***	****	Strong	Strong
Decreased reproductive function in Baltic seals	PCBs	***	**	***	***	****	Strong	Moderate
Great Lakes embryo mortality and edema syndrome in birds	PCHs, PCBs	****	****	****	****	****	Strong	Weak
Eggshell thinning in colonial waterbirds	DDE and other DDT metabolites	****	****	****	***	****	Strong	Moderate
Reproductive abnormalities in Lake Apopka alligators	Dicofol and agricultural pesticides	****	***	***	***	**	Moderate	Moderate
Developmental abnormalities and reproductive failure in Lake Ontario trout	Dioxins and co-planar PCBs	****	****	***	****	****	Strong	Weak
Vitellogenin induction in fish exposed to sewage treatment plants in England	Estrogenic contaminants	****	****	***	****	**	Strong	Strong
Reproductive alterations in fish exposed to bleached kraft mill effluent in Ontario	Bleached kraft mill effluent (BKME)	****	****	***	****	***	Strong	Strong

Adverse effect vs. mechanism of action

Endocrine disruption is not an adverse effect per se, but rather a potential mechanism of action or descriptor of functional change that may lead to adverse health effects as measured by well-established endpoints such as reproductive toxicity or impaired development [2,10]. The Weybridge definition of "endocrine disruption" encompasses both the endocrine mechanism of action and adverse health effects (i.e., "an endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function" [5]). This definition implicitly recognizes that, while substances may have the potential to interact with the endocrine system, they do not necessarily adversely affect wildlife or ecosystem health. Evidence that a substance interacts with a component of the endocrine system through a particular mechanism does not provide any information on whether that substance causes other biological changes, particularly adverse health effects. By adopting a definition of endocrine disruption that includes evidence of adverse effects and not just evidence of a potential for interaction with the endocrine system, the focus is on understanding what is most important to protect the environment.

Structure and function of ecosystems

In ecological risk assessment, population-level effects take precedent over individual-level effects [11,12]. Within the context of endocrine disruption, the CSTEE [5] has similarly emphasized that ecological risk assessment is intended to evaluate risk of effects on the structure and function of ecosystems. Endocrine alterations at the individual level may or may not result in consequences on populations, communities, or ecosystems. A suitable strategy must therefore focus on endpoints relevant to the detection of population- or community-level effects. Understanding the mechanism is therefore secondary to ecological effects that can elucidate effects on populations, communities, and ecosystems [13]. Ecological effects are those that manifest themselves at the population level or higher. The most common unit of study in the population and endpoints of concern are those related to population size or reproductive capacity. Higher levels of organization include the community (an assemblage of species) and the ecosystem (communities within their physical and biological environment). Population-level effects are more difficult to detect than effects on individuals due to background variability. Population-level measures include size, age structure, sex ratios, recruitment, and biomass. Other measures are used for communities (i.e., keystone species, sentinel species, predator number, and predator–prey ratio) or ecosystems (i.e., species diversity, species richness, species abundance, species assemblage, primary production, biomass change, nutrient cycling, and energy flow). These require even greater interpretation before cause–effect relationships can be demonstrated. A variety of potential pyramid effects at the community or ecosystem level have been identified [13]. They include bioaccumulation, biomagnification, cascading effects (effects following from one effect on a component of the ecosystem), keystone effects (changes in population size of species linked with a community such as predator-prey), and matrix effects (those occurring in adjacent communities or ecosystems by virtue of proximity).

Tiered hazard assessment

Screening assays should be short-term, rapid, and inexpensive assays designed to detect specific hormonal activity. They should be based on a discrete mode or mechanism of interaction with the endocrine system. Screening assays are designed to detect substances that have the potential to interact with one or more components of the endocrine system. They do not detect adverse effects per se and cannot be interpreted as short-term and predictive tests for hazard assessment purposes. Positive results in a screening assay/battery (based upon a weight of evidence evaluation) indicate a high priority for evaluation in a definitive test. Hazard assessment for risk characterization must rely primarily on the results

of definitive tests. This is not to say that screening assays are used only to prioritize substances for further testing, but their use in hazard identification and risk characterization should be constrained to providing mechanistic clarification regarding adverse effects demonstrated by definitive tests. Longer-term in vivo tests (e.g., mammalian reproduction studies, fish life-cycle tests) should be employed to identify adverse effects using relevant, atypical endpoints. Dose–response characterization should also be a goal of such tests. Hazard identification for risk characterization should rely primarily on the results of definitive tests. Some sources have implied that "endocrine disruption" is a new and previously unknown type of toxicity, and that current toxicological testing cannot address such potential effects. This is clearly not the case. Work is in progress via the Organization for Economic Cooperation and Development (OECD) Endocrine Disruptor Testing and Assessment (EDTA) Work Group to review and, where appropriate, change/introduce screening and testing protocols. Indeed, this is an explicit recommendation of the CSTEE [5].

Hutchinson et al. [7] provide a clear line of reasoning that the potential for exposure should drive the selection of appropriate test organisms for hazard assessment. Part of the international OECD effort is to nominate, discuss, and reach consensus on a practical number of representative taxonomic groups and specific species within each group to employ in screens and tests to investigate the potential hazard of EASs. Hazard identification is an assessment of the qualitative toxicity of a chemical, and constitutes an initial step in the risk characterization process. A defensible hazard characterization for hormonally active chemicals requires not only summarizing toxicological screening and testing data (hazard identification), but also an objective evaluation of whether the effects produced are adverse and whether adverse effects are due to a hormonal activity of the chemical. A "weight of evidence" (WoE) evaluation is an objective and balanced interpretation of the totality of scientific evidence regarding hormonal activity and adverse effects that might result from an endocrine mechanism. In conducting a WoE evaluation, a tiered, hierarchical approach is recommended to guide development and use of screens and tests for hormonally active agents. In such a tiered approach, results from definitive tests must outweigh or supersede results from screening assays. An example of such a WoE framework is provided in the Endocrine Modulation Steering Group (EMSG), [14]. The principles presented in this approach are based on "sound evaluation practices and are in line with CSTEE approaches to chemical hazard and risk assessment" [10]. A concrete example of the application of a WoE approach to wildlife with the context of endocrine disruption is that of Johnson [15].

The Weybridge [5] workshop recommended the development of a hazard identification and characterization strategy for endocrine modulation that involved three broad stages or tiers:

- **Initial assessment.** Assemble and use available information (e.g., data on toxicity) to gather evidence on whether a chemical may or may not lead to adverse effects associated with endocrine modulation or use other available information (e.g., structure–activity relationships [SARs]) to gather evidence on whether a chemical may or may not take part in modes of action underlying endocrine modulation (e.g., receptor binding). For the purpose of prioritization.
- **Screening.** Use in vitro and in vivo short-term assays to assess whether a chemical could act via a specific mode or mechanism of action that may lead to endocrine modulation. It was envisioned that in vitro assays could precede in vivo assays. However, the results of in vivo screening assays were deemed essential before proceeding to the testing tier to consider absorption, metabolism, etc.
- **Testing.** Enhanced short-term (subchronic) and long-term (chronic) test guidelines would be employed to determine whether a chemical elicited a particular hazard or adverse effect(s) and would characterize the dose–response relationship for any adverse effect(s) that were observed.

A tiered hierarchical framework provides the most efficient, and therefore protective, mechanism to obtain, organize, and interpret necessary data. The tiered approach uses available information and/or less resource-intensive screening to determine the extent to which substances warrant more resource-intensive testing. Such an approach allows for optimal use of finite resources (which includes the use

of laboratory animals, laboratory capacity, and financial resources) and is necessary to expeditiously evaluate chemicals of greatest concern [7].

Test methodologies

The CSTEE [2] emphasized that ecological risk assessment is intended to evaluate risks on the structure and function of ecosystems. Ecotoxicity testing must therefore focus on relevant endpoints for the detection of population–community effects (i.e., reproduction and development). All laboratory toxicity tests should be standardized and validated in advance of their use in a regulatory testing program to assess endocrine activity. Use of standardized and validated toxicity methods facilitates scientific interpretation of study results, promotes clear and consistent risk assessment analyses, and enhances confidence in the use of test results for protection of wildlife. When new toxicity testing is planned, the study protocols should follow globally harmonized guidelines to promote mutual acceptance of data, and thus preclude additional and repetitive testing at a later date. That said, some concerns have been expressed regarding the ability of low-tier-level tests in the detection of ecological risk of endocrine disruptors [2]. Concerns relate to the suitability of test species (i.e., use of parthenogenic organisms such as *Daphnia*) and epistemological uncertainties when extrapolating from acute lethality to long-term effects [2]. This is problematic, as risk assessment follows a tiered approach. Higher-tier tests are only employed when low-tier evaluations indicate potentially unacceptable risks. Hence, the concern regarding the capability of low-level tiers to detect ecological risk from endocrine disruptors. The CSTEE [2] concluded that existing test guidelines cannot detect all endocrine-disrupting effects, and that current test guidelines have to be enhanced or new guidelines developed, preferably via international cooperation to avoid duplication (e.g., OECD). Reliance on in vitro assays for predicting in vivo, EAS-mediated effects was not recommended as they were deemed to be prone to false negatives and false positives. Major emphasis was placed on in vivo assays. Priority was placed on establishing long-term tests to detect ecologically relevant effects related to endocrine disruption. This includes the enhancement of the OECD 407 repeated oral toxicity and OECD 416 reproduction toxicity mammalian tests, development of enhanced early-life stage (ELS) and partial fish life-cycle studies, enhancement of existing avian tests, and development of appropriate invertebrate test systems. In many instances, industry has provided laboratory resources to assist in the development of assay validation data [16–18].

The foundation of the OECD framework for evaluating potential EASs will make use of standardized, validated, and internationally harmonized test methods. However, as basic research on endocrine mechanisms advance, new and novel scientific methods have and will continue to be reported. These new and novel types of studies are significantly different from laboratory studies using standardized and validated techniques. Research laboratory studies reporting novel test methods, nonstandardized and nonvalidated methods, or nonstandard test species may lack quality criteria for regulatory purposes (i.e., reliability of test methods, relevance/significance of endpoints). Under such circumstances, such studies should not be used for regulatory action per se, but rather trigger further testing and/or method validation efforts. Recognizing that new and novel methods and studies with nonstandard species may provide important scientific information, it is clear that they cannot be ignored. The preferred action would be to evaluate the substance of concern in one of the wide variety of existing validated test methods using standardized OECD methods and species (or similarly validated scientific methods, for example, those promulgated by the International Standards Organization [ISO], ASTM International [ASTM], or the U. S. Environmental Protection Agency [USEPA]). Alternatively, the new or novel test method could be subjected to standardization and validation within the OECD EDTA program or within a similar formal program if the method is viewed as necessary to augment or replace (an) existing test method(s) in the screening and testing battery. For a new or revised test method to be considered validated for regulatory purposes, it should meet criteria such as those specified by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM; see <http://iccvam.niehs.nih.gov/docs/guidelines/criteria.htm>).

Exposure assessment

It is important to keep in mind that hazard identification and characterization alone are still insufficient to characterize risk. Likewise, exposure per se does not constitute an adverse effect. Risk characterization requires integration of scientific data and knowledge relating to both hazard and exposure. It is therefore important to evaluate if a compound is present in the environment. If the compound is not present or the ecological systems of interest are not exposed, then it can be concluded that the agent is not a factor in the system. There can be no risk without exposure. Risk only occurs when a threshold corresponding to a no-effect concentration (at the population level) is exceeded. The corollary is that for all substances there are exposures below which harm will not occur. Acceptance of this fact is important as advances in analytical techniques mean that an ever-increasing number of natural and anthropogenic compounds are being detected in the environment via an expanding array of increasingly sensitive techniques. For example, within the aquatic environment contaminants are now frequently reported in the ng/l range or even lower. It will be impractical, and in many cases undesirable, to ban all such compounds purely on the basis of identified presence in the environment. There is also a question of scale of risk to be considered. Is exposure localized or widespread? This has obvious implications for any potential chemical management strategies. Within the context of endocrine disruption, it is possible that potential effects are separated in time from critical exposures. Impacts upon adult development may theoretically be dependent upon exposures in an earlier (more sensitive) life phase. This gives rise to the concept of critical windows of exposure and the need to consider temporal as well as spatial aspects in exposure assessment.

Relative potency

An important consideration is whether the critical toxic mechanism for a particular substance is via endocrine disruption (i.e., what is the probability of achieving an effective exposure that elicits an endocrine-mediated effect relative to the probability of inducing prior major systemic toxicity via an alternative mechanism) [19]. From a pragmatic perspective, substances should not be regulated on the basis of potential endocrine effects that only occur at extreme doses above those incurring other critical toxicity. This approach has similarly been proposed by the CSTEE [10]. They state that only in situations where the endocrine-disrupting effects are critical (i.e., the most potent) in comparison to other toxic hazards, should the endocrine disruption be considered for hazard and risk assessments: “Little importance should be assigned to situations where the no-observed effect level for endocrine disruption is substantially higher than that for other adverse effects caused by a chemical”.

Risk/benefit

Any decision to manage compounds on the basis of purported endocrine hazard should examine potential benefits, and costs of and lack of action to human or wildlife health. The scope of such considerations should not be restricted to economic considerations alone. Cost/benefit analyses feature as one of the general principles of application in the European Commission (EC) communication on the precautionary principle [20]. There may be circumstances where zero emissions may be technically infeasible, and perhaps not even desirable, as it may entail loss of critical benefits or imply consumption of alternative economic or material resources that will also carry societal or environmental costs. For example, natural and synthetic estrogens are consistently believed responsible for the majority of the estrogenic effects observed in fish populations near sewage treatment plants [21,22]. Yet, the loss of benefit associated with a ban on ethinyl estradiol used in the contraceptive pill is an example of a critical benefit that society is not prepared to countenance. Risk management of effects in fish populations from steroids in sewage effluent would therefore be limited to the imposition of improved sewage treat-

ment technologies rather than an outright ban on use per se. This relative balance of risk and loss of benefit needs to be considered in any risk management deliberations.

Effects benchmarking

Several compounds (e.g., nonylphenol) have been identified as being estrogenic in fish. Such observations have led to calls for such compounds to be banned on the basis of their potential hazard to fish populations in watercourses impacted by domestic wastewater treatment plant effluent discharges. This ignores the likely fact that the large majority of the estrogenic potential of sewage influent is undoubtedly attributable to natural and synthetic sex steroids such as estradiol, estrone, and ethinyl estradiol [21,22]. Under such circumstances, management actions on nonylphenol—which has impacts on a local level—would not have addressed the causal factor for the large majority of impacted watercourses where widespread exposure to steroids is more critical. The problem would have remained, as causality would not have been affected. Within the environment, the risk from substances with common modes of action should therefore be considered collectively and compared with likely background concentrations of naturally occurring but similarly acting compounds. Any distinction between the two is artificial [10]. Likewise, the impacts of exogenous agents should be benchmarked against endogenously occurring compounds with a similar mode of action (e.g., exposure to xenoestrogens via the environment should be benchmarked against endogenous steroid levels) or other exogenous agents of natural origin such as dietary phytoestrogens. Consistent with this stance is the fact that one of the general principles of application in the EC communication on the precautionary principle [20] is that of *nondiscrimination* (i.e., "comparable situations should not be treated differently").

FUTURE RESEARCH ADDRESSING RISK FROM ENDOCRINE DISRUPTORS

Specific research recommendations identified in the IPCS-GAED report (IPCS, 2002) are highlighted below (Table 4). These demonstrate the current incomplete understanding of the issue and the need to address uncertainties. Many of the points identified reflect earlier similar recommendations by the likes of the CSTEE [2] or the National Research Council (NRC) [13]. Also presented are details of the current LRI-funded/cofunded research program that are relevant to the specific research recommendations. These projects reflect attempts by industry to reduce uncertainty surrounding the risk from EASs to wildlife and include consideration of issues such as mixtures. Full details, including lists of publications that have arisen from this research, are provided on the LRI Web sites.

Table 4 IPCS research areas and LRI activities.

Biology underlying endocrine-mediated effects
• Expand basic knowledge about endocrine systems in wildlife.
• Elucidate the range of mechanism by which endocrine disruption may interfere with reproductive/population success, immune function, neurobehavioral, and development of cancer, at all levels of biological organization and at key stages of life cycles.
Relevant LRI-sponsored research (lead researcher and institute):
- Genetic sex determination of fish (R. Devlin, Fisheries and Oceans, Canada)
- Estrogens and neuro-endocrine regulation of reproduction in fish (H. Goos, University of Utrecht, Netherlands)
- Aquatic invertebrates (L. Pinder, NERC Institute of Freshwater Ecology, UK)
- Effects of DES on the fetus considering its placental transport (N. Utoguchi, Teikyo University, Japan)
- Molecular and cellular approach on the reproductive tract abnormalities induced by prenatal exposure to DES in mice (T. Nagao, Hatano Research Institute, Japan)

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Table 4 (*Continued*).

- Relationship between the effects to reproduction and vitellogenin induction of fish by EDCs (J. Koyama, Kagosima University, Japan)
- Toxicological significance of vitellogenin synthesis induced by estrogens exogenously administered in the inbred and wild medaka (S. Hamaguchi, Niigata University, Japan)
- A whole lake experiment to examine the effects of a synthetic estrogen on aquatic populations (K. Kidd, Environment Canada)

Methodology

- Develop improved methodologies to assess dose-response relationships at environmentally relevant concentrations.
- Develop more specific and sensitive biomarkers for detecting endocrine-mediated effects in individuals and populations.

  Relevant LRI-sponsored research (lead researcher and institute):
  - Fish 14-day Vitellogenin Screening Assay TG Screen (T. Hutchinson, Astra-Zeneca Brixham Environmental Laboratory, UK)
  - Fish extended early life stage and reproductive chronic assays (T. Hutchinson, Astra-Zeneca Brixham Environmental Laboratory, UK and R. Bogers, NOTOX, NL)
  - Endocrine disruption in the marine environment – EDMAR (P. Matthiessen, CEFAS, UK)
  - Endocrine disruption in the aquatic environment: Laboratory investigations of endocrine active chemicals (J. Sumpter, Brunel University, UK)
  - Development, validation, and application of in vitro and in vivo test systems for nonestrogenic EDCs in wildlife—especially amphibians (A. Murk, University of Wageningen, Netherlands)
  - Avian two-generation toxicity test development (I. Chahoud, Free University of Berlin, Germany)
  - Validation of OECD test guidelines for the uterotrophic, Hershberger, and enhanced TG 407 assays (EMSG industry consortium)
  - Field-deployable methods for measuring endocrine-mediated modulation of songbird reproduction (L. Brewer, Springborn Laboratories, USA and A. Fairbrother, Parametrix, USA)
  - Development, application, and validation of a sheepshead minnow estrogen-responsive cDNA microarray (M. Hemmer and L. Folmar, NHEERL, USEPA)
  - Evaluation of eastern fence lizards (*Sceloporus undulatus*) and western fence lizards (*S. occidentalis*) as reptile models for assessment of endocrine mediated toxicity (L. Talent and D. Janz, Oklahoma State University, USA)
  - Avian one-generation reproductive study with Japanese quail incorporating endocrine endpoints (L. Brewer, Spingborn Laboratories, USA)
  - Effect of EDCs on the development of the cerebral nerve system (R. Kishi, Hokkaido University, Japan)

Monitoring

- Increase long-term monitoring of "sentinel" wildlife species to provide baseline data on population status.
- Improve international collaboration and cooperative research to assess the exposure and effects of EDCs on wildlife populations on a more global basis.

  Relevant LRI-sponsored research (lead researcher and institute):
  - Environmental effects on uterine tissues of Baltic Seals with special emphasis on organochlorins and uterine Leimyomas (M. Olssen, Swedish Natural History Museum, Sweden)
  - Examination of bull sperm as a sentinel species for male reproductive health (J. Van Os, Global Institute for Study of Natural Resources, Netherlands)

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Table 4 (*Continued*).

Identifying Endocrine Disruptors
• Continue to identify chemicals (persistent and nonpersistent, naturally occurring, and anthropogenic) that are the most likely candidates for high-impact effects in populations at environmentally relevant concentrations. • Identify "hotspots" for exposure or effects that warrant particular concern. • Focus work on populations/subgroups most likely to be susceptible to endocrine disruptors. • Assess the role of endocrine disruptors relative to other environmental stressors on the fitness of the population. **Relevant LRI-sponsored research (lead researcher and institute):** - Endocrine-modulating effects in fish along the Elbe River and in reference areas. Assessment of risks related to habitat conditions and the natural variability of endocrine functions (L. Karbe, University of Hamburg, Germany) - Ecological hazard assessment for endocrine toxicity of chemical mixtures (C. Borgert and T. Gross, USGS/University of Florida, USA)
Database Development
• Develop better global data, especially in countries outside North America and Europe, on status and trends of environmental contamination, exposure, and health outcomes. • Improve international coordination for sharing information on effects caused by endocrine disruption. **Relevant LRI-sponsored research (lead researcher and institute):** - Studies on actual conditions in relation to the influences of chemical substances in Japanese wild animals (M. Uchiyama, Toyama University, Japan)

DISCUSSION

Most legal frameworks require regulators to demonstrate that under certain defined conditions, a substance poses an unacceptable risk. Implicit in the application of the precautionary principle is a shift of the so-called "burden of proof" from regulatory agencies to demonstrate risk to manufacturers or formulators to demonstrate that no harm to the environment will occur. This reversal of the burden of proof ignores the scientific fact that it is always impossible to prove a negative; thus, it can never be proven that any given activity or substance will pose no harm to the environment [23]. The claim that a particular chemical causes adverse effects in the environment is feasible in principle to evaluate. One example is sufficient to prove the claim and supply the burden of proof. The opposing claim that the chemical does not cause adverse effects in the environment can never be lifted. Situations will exist that cannot be adequately covered by investigations. Such a claim can therefore only be evaluated by induction. The better and more comprehensive the investigation, the more likely the claim, but there can never be complete certainty. Only example would falsify the claim. This restriction on lifting the burden of proof applies to all chemicals regardless of intrinsic hazardous properties. Environmental acceptance of any given compound can therefore only be demonstrated by some degree of induction and reasonable likelihood according to acceptable criteria [23]. Zero-risk and absolute safety can never be achieved or proven for any issue or substance. It is consequently a question of confidence or acceptability of whether uncertainty has been reduced sufficiently. The maximum that can be done is to provide evidence that the activity or substance in question has been adequately tested and evaluated against potential known effects. This has always been the stance of the chemical industry. It is also reflected somewhat in the EC communication on the precautionary principle [20]. Within the context of the so-called general principles of application under *proportionality*, there is an explicit statement that "measures based on the precautionary principle…must not aim at zero risk, something which rarely exists." In other words, there must be a proportional response to a potential risk.

Environmental nonacceptance implies some form of risk management. Substitution of a compound requires a full evaluation of all candidate substitute compounds. All will carry economic, socie-

tal, and environmental costs as well as benefits. Perception of the relative balance of these will be very different for each stakeholder. The societal process of the appreciation and perception of risk does not necessarily make use of a rational and technically informed procedure. Judgment is based on personal impression founded on a limited and unstructured subset of information with qualitative rather than quantitative attributes. The scientific process of risk assessment is more formally structured and subject to standardized, rational, and scientifically accepted rules and procedures. In contrast, risk perception is not dependent upon a formal evaluation scheme, but on a large number of subjective variables (e.g., education, natural/social environment, ethical, political and religious beliefs, physical and mental health). Herein lies a major problem with the use of the precautionary principle in this context. All regulatory decisions need to take place within a transparent and predictable framework, otherwise all actions such as substitution are precluded, as the acceptability of any alternative outcome to all stakeholders will be unpredictable and not apparent. Without such predictability and transparency, any decision framework may be seen as an arbitrary black-box process. Given these restrictions, risk assessment must provide the foundation for effective regulation. Other approaches potentially lead to arbitrary regulatory decisions.

Where risk assessment cannot be conducted with appropriate certainty, the precautionary principle may be invoked, particularly where effects are perceived as being potentially serious and/or irreversible. This implicit primacy of risk assessment is reflected in the EC communication on the precautionary principle [20]. Use of the precautionary principle as a management strategy is always preceded by an initial evaluation of risk. The application of the precautionary principle in this way therefore assumes that potentially adverse effects have been identified and that scientific evaluation does not allow the determination of risk with sufficient certainty. Given that scientific knowledge is not static, the degree of certainty may be subject to future revision. Industry would therefore also suggest that any use of the precautionary principle is potentially transitory pending other data (i.e., subject to review). This is one of the general principles of application in the EC communication [20]. It states that precautionary "measures should be maintained as long as the scientific data are inadequate, imprecise, or inconclusive." Measures are therefore provisional pending the availability of more reliable data.

One of the problems with ecological risk assessment is that the focus is on the effects on the structure and function of the ecosystem, a difficult concept to relate. The assessment process itself is complex, requires sophisticated tools, and is difficult to communicate. As well as communicating risks, it is also important to describe benefits so that there can be a fair and factually oriented debate between stakeholders. In the first instance, the provision of factual scientific information to stakeholders is important. Explaining assessment tools and results will aid understanding of risks. Secondly, participation is important. All stakeholders should be empowered to express their views to allow productive debate. This is essential to establish trust and social responsibility. The public will judge all scientists from regulators, NGOs, and industry on their combined capacity to manage issues together in a timely fashion.

Regardless of the decision basis (i.e., hazard or risk), there are certain common research needs. Within the context of endocrine disruption, the LRI has attempted to address gaps in knowledge and data via collaborative and cooperative projects. Much work has been sponsored dealing with the development and validation of mammalian and environmental effects testing methodologies that can be used for comparative hazard or risk assessment. Exposure data is similarly required when assessing risk or causal relationships between exposure and wildlife health effects. This includes sources, fate and transport processes, transformation, and degradation. This is also a focus of LRI-sponsored research. Great emphasis has been placed on publication and sharing of data with all stakeholders for informed policy and regulatory decision making based on best available scientific knowledge. Any instances where there is an overestimation of risk will be due to an ignorance or lack of the technical facts leading to uncertainty. The solution to such conflict is to reduce ignorance through the provision of data on toxicity, exposure routes, and effects of environmental contaminants, thereby contributing to an informed decision-making process. This is the central aim of the LRI.

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