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Annual Review

WILDLIFE AND ENDOCRINE DISRUPTERS: REQUIREMENTS FOR HAZARD IDENTIFICATION

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Abstract—The increasing demands of the global economy and our growing human population exert profound effects on the ecosystem. As a species, we have an essential need for quality air, water, and soil and a diversity of living organisms, so any vision of a sustainable future must be formulated on the basis of their protection. Decisions on the most effective ways to protect ecosystems must be prioritized on the basis of science and our understanding of the most important factors that affect wildlife populations. In the case of endocrine disrupters, it is important that further research be conducted (1) to determine the status and trends of wildlife populations and the evidence for a widespread effect of endocrine disrupters, (2) to determine the extent to which species differences in the mechanisms that control reproduction and development will affect their sensitivity to endocrine disrupters, and (3) to ensure full validation and refinement of new test methods for assessing the effects of endocrine disrupters across diverse species. Premature implementation of testing and assessment methods for endocrine disrupters, without appropriate consideration of the above points, may result in assessment schemes that placate public opinion but neither alleviate the major factors affecting wildlife populations nor protect diverse species with distinctive endocrine systems.

Keywords—Endocrine disruption Wildlife Hazard identification

INTRODUCTION

The possibility that wildlife populations have been affected by exposure to endocrine-disrupting compounds has stimulated intense debate. For the purposes of the following discussion, an endocrine disrupter is defined as an exogenous substance that causes adverse health effects in an intact organism or its progeny consequent to changes in endocrine function [1]. Numerous working groups, at both the national and international levels, have been formed to evaluate the plausibility and extent of the evidence and the need for specific regulations. The European Centre for Ecotoxicology and Toxicology of Chemicals convened a task force to study this important problem, to prioritize research areas to advance science in this area, and to advise toxicologists and ecotoxicologists on appropriate testing methods and hazard identification strategies. Within this task force, a subgroup was formed to concentrate on the aspects of this debate that were specific to wildlife. Considering that the U.S. Environmental Protection Agency has been mandated to implement testing strategies for endocrine disrupters within a very short time frame [2,3], with the intention to assess the potential of chemicals to produce endocrine-mediated effects in humans and wildlife, we believe that further critical evaluation of the evidence for widespread effects in wildlife is required. In addition, interspecies differences in endocrine systems and function, how these may affect potential test utility, and the effectiveness of the resultant assessment strategies need to be considered. These points are discussed in the context of a potential hazard identification strategy. Recommendations for research that identify the need to advance our knowledge of the potential effects in wildlife, to conduct further research on the comparative endocrinology of wildlife species, and to improve available hazard identification methods are also proposed. These factors must be considered before testing strategies for endocrine disrupters are implemented since the objective should be to protect the health of the ecosystem using scientifically sound principles.

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SIGNIFICANCE OF ENDOCRINE DISRUPTERS FOR WILDLIFE POPULATIONS

Laboratory studies, conducted as early as the first half of this century, have indicated that exposure to hormonally active agents can affect the reproductive development of some wildlife species [4]. In recent years, attention has turned to the potential for such effects in wildlife populations [5–7]. A significant number of studies have demonstrated reproductive and developmental abnormalities in various groups of organisms, including birds, fish, invertebrates, and reptiles, with special concern for wildlife species susceptible to exposure to endocrine disrupters in or via the aquatic environment. From an ecological perspective, however, it is currently difficult to assess the true scale of such health problems in wildlife populations owing to a paucity of baseline ecological information. The complexity of this question is illustrated by the observation that some wildlife populations are growing in number despite clear localized evidence of adverse morphological and biochemical effects plausibly caused by exposure to endocrine disrupters. Perhaps the most topical example is the North American alligator, *Alligator mississippiensis*, populations of which have been intensively studied in Florida, USA. In this instance, convincing evidence has been reported of endocrine disruption in alligators following gross pollution of Lake Apopka by organochlorine compounds [8,9]. However, the

overall population of *A. mississippiensis* in the southeastern United States appears to be growing in number. Indeed, from 1974 to 1992, the density of alligators increased by 41% in Florida [10]. The dramatic recovery of this once vulnerable species, as a direct result of reduced hunting pressure and other conservation efforts, has resulted in its ceasing to be classified as an endangered species [11]. These observations are juxtaposed to emphasize that even though there is understandable concern regarding the effects of endocrine disrupters in reptiles in pollution "hot spots" such as Lake Apopka, and that uncontrolled release of such chemicals can undoubtedly result in adverse effects and such areas obviously require urgent remediation, there is a need to consider such observations in the context of other relevant ecosystems.

As a second example, there is also concern that freshwater fish species may be affected in some parts of North America by endocrine disrupters [12]. The concern that such organisms are under threat is understandable since some 3% of freshwater species in North and South America have become extinct in the last 100 years [13]. In North America, it is recognized that the predominant factors causing declines in fish species are habitat destruction (affecting 73% of species) and displacement by introduced species (affecting 68% of species) [13]. For perspective, alteration of habitats by chemical pollutants is considered to have affected some 38% of species [13]. These figures add up to more than 100% because more than one agent impinges on many fish populations. The forces causing dramatic declines in some fish species on a global scale also include overexploitation and climatic factors [14–16]. Such observations highlight the need to understand the relative ecological hazard of endocrine disrupters in comparison to other anthropogenic activities.

In Europe it is recognized that many rivers flowing through urban and industrial developments were historically grossly polluted. In recent decades, however, substantial improvements have been made in the treatment and reduction of domestic and industrial wastes previously discharged into these rivers with consequent ecosystem recovery. In the United Kingdom, for instance, the biological and chemical quality of rivers improved in almost 26% of river lengths between 1990 and 1995 [17]. At the same time, however, there is evidence that fish from some U.K. rivers exhibit signs of estrogenic effects [18]. Recent evidence has indicated that these effects are due predominantly to the presence of natural estrogens in municipal sewage effluent [19], although in some cases alkyl phenolic compounds have also been implicated [20]. As with North American alligators, there is a need to consider objectively all available evidence on ecosystem health to ensure that appropriate measures are taken and that wildlife populations are effectively protected as a result. This is essential if environmental decision makers are to direct society's finite resources to provide maximal environmental benefit.

To facilitate an objective debate, it is important that a database be established to collate the evidence, or otherwise, for potential widespread endocrine disruption and associated reproductive impairment in wildlife. The availability of such integrated information on ecological trends is as fundamentally important to environmental management as human epidemiology is to medicine. Indeed, some valuable progress has been made in this area. For example, the recent U.S. publication entitled "Our Living Resources" [10] collates evidence indicating that there have been particularly serious declines in North American populations of grassland and scrubland birds, amphibians, fish, and aquatic invertebrates, especially freshwater mussels. It is not known whether endocrine disrupters are involved in such population declines, and research is urgently needed to elucidate possible causal factors, including chemical contamination, habitat destruction, introduction of nonnative species, etc.

An ecological risk assessment of the potential effects of endocrine disruptor in wildlife should pay particular attention to the fundamental factors that dictate ecological sustainability. In this context, a paradigm of evolutionary biology is that, under optimal environmental conditions, there is a reproductive surplus in a population and that the consequent competition between individuals drives the process of natural selection [21]. Natural selection therefore nurtures the individuals with the greatest fitness, namely the individuals with the ability to reproduce and survive. Protection of these essential parameters should be the fundamental goal of environmental (ecological) risk assessment for endocrine disrupters and other potential chemical contaminants. Indeed, it is a measure of the recognition of the ecological importance of the reproduction and survival endpoints that they are already included in many existing protocols for evaluating the toxicity of chemicals to wildlife [22]. The precise endocrine mechanisms that influence the reproductive success of a species are diverse and are affected by a range of physicochemical and biological factors. The fundamental importance of individual reproduction in assimilating the effects of many diverse environmental factors is represented in Figure 1. These diverse environmental factors include xenoestrogens and other chemicals observed to affect the reproductive physiology of fish and other wildlife species [8, 23–25]. It is also recognized that environmental stress can have affect the survival and immunocompetance of fish populations [26,27] and that such stress can also impair the reproductive success of aquatic species [28–30]. In addition, nutritional deficiencies may have profound impacts on the survival, development, and reproductive health of aquatic organisms [31,32]. An important example of this complex phenomenon is the fact that "fry mortality syndrome," seen in Baltic salmon species, can be treated effectively by feeding thiamine [33]. Therefore, there is no doubt that a variety of factors impinge upon the reproductive output and fitness of natural populations. To ensure that appropriate actions are taken to protect the global environment, the debate on endocrine disruption in wildlife needs to broaden and to take further account of the ecological dimension.

SPECIES DIFFERENCES IN SEX DETERMINATION AND COMPARATIVE ENDOCRINOLOGY

Interspecies differences that may affect sensitivity and response to endocrine disrupters include quantitative and qualitative variability in endogenous hormone and receptor levels, differences in the timing and duration of critical periods of development, and interspecies differences in the sex-determination process. Pharmacokinetic differences may also affect the concentration at the target site, pharmacodynamic differences in the interactions with the molecular target, and differences in subsequent responses. Interspecies genomic differences in, for example, hormone response elements and the related complement of hormone-activated genes will also play an important role. It is important to note that few species of wildlife have been studied in any depth, and there is a paucity of even basic endocrinology data for most species. As an illustration, of species given scientific names, fewer than 10%

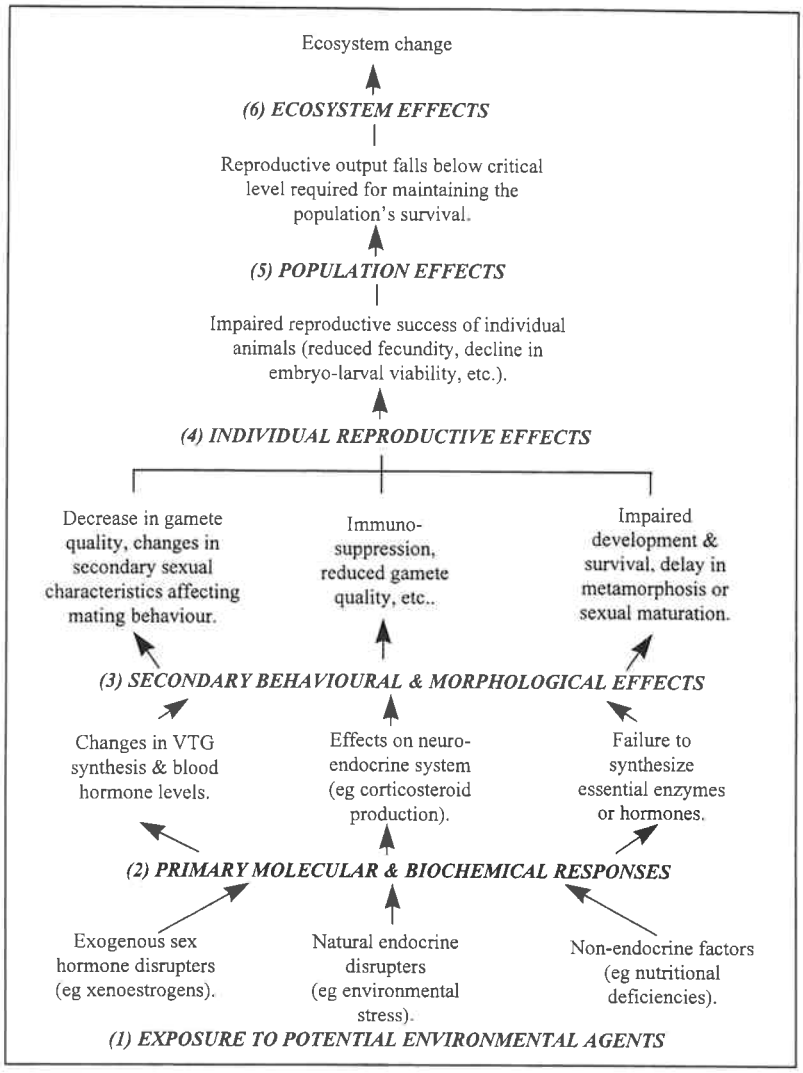

Fig. 1. Mechanisms by which endocrine disrupters and other environmental agents affect the reproductive health and survival of aquatic wildlife populations.

have been studied at a level deeper than gross anatomy [13]. These factors should be considered in the context of endocrine disrupters and potential test methods, because these may have profound impacts on test species selection and understanding of ecological effects. The proposition that novel biomarkers or in vitro methods may be incorporated into a wildlife testing and assessment strategy for endocrine disrupters requires in-depth consideration of exactly how the results of such tests will be interpreted and to what extent the results are predictive of adverse health effects in wildlife. Although lower-tier tests may be useful for screening purposes and for prioritization of chemicals for testing, it should be recognized that the information from such tests is limited, not only for predicting whole organism effects but also because species differences may limit the extent to which the results of such tests can be extrapolated to higher-tier effects in other species. Hence, it is vital that lower-tier tests be used and interpreted in the correct context and that species differences be considered when attempts are made to use these screening tests in a tiered hazard identification strategy.

Sex determination in wildlife

Significant variability exists between wildlife species in the mechanisms that control sex determination (Fig. 2). For the vast majority of species, virtually nothing is known about the molecular or physiological basis of this process. Sexual differentiation depends on chromosome constitution and is controlled by different hormones in different species. It is noteworthy that the sex-determining gene in mammals (SRY), the most intensively studied taxonomic group, was characterized only recently [34]. No equivalent sex-determining gene has been characterized in any other taxonomic group. However, some sex-linked genetic markers whose gene products are not directly involved in sex differentiation are available for a limited number of fish [35,36] and avian species [37]. In contrast to mammals, in which the default sex is female (XX), the default sex in birds is male (ZZ), and avian male genotypes may be feminized by exposure to estrogens during embryogenesis [24]. The genetic basis of sex determination in many species of fish is largely uncharacterized; however, present evidence indicates that a variety of sex-determination mechanisms exist in fish, including polyfactorial systems, male and female heterogamety, hermaphrodism, and environmental sex determination [38]. Important differences in hormonal control of sexual differentiation exist between species since this process is primarily controlled by androgens in mammals, by androgens and estrogens in reptiles, and by estrogens in other

Taxonomic group	Genetic sex determination		Environmental sex determination		Maternal sex determination
	XX/XY F/M	ZZ/ZW M/F	Temperature	Other factors	
Invertebrates:					
Annelids				*FP	*
Crustaceans				*PH	
Insects	*		*		*
Molluscs				*PF	
Vertebrates:					
Amphibians	*	*	*		
Birds		*			
Fish	*	*	*	*SH	
Mammals	*				
Reptiles	*	*	*		

Fig. 2. The diversity of sex-determining mechanisms in wildlife. F, female; M, male; FP, female proximity; PF, pair formation; PH, photoperiod; SH, social hierarchy. Adapted from Barnes et al. [66] and Pieu [67].

vertebrates, such as birds; hence, the relative effects of exposure to endocrine disrupters during this stage of development may well be significantly different between species. For example, it is expected that androgen analogues would be more likely to have an effect on the sexual differentiation of mammal, rather than avian, species [24]. In addition, there is little understanding of the mechanisms underlying temperature-dependent sex determination exhibited by some species, although it is believed that direct temperature effects on steroidogenic enzymes can play a role.

This variability between species and our substantial lack of basic understanding will have consequences for interpretation of the results of field studies and the selection of appropriate test species. For example, some species of birds, such as the Seychelles warbler, can manipulate the sex ratio of their offspring such that predominantly males or females are produced by a breeding pair, depending on the availability of food [39]. The Seychelles warbler is an extreme example because this species can switch from producing up to 77% males when food availability is low to producing as few as 13% males when food availability is high. In contrast, most other bird species that have been studied in this regard exhibit differences in offspring sex ratio of $<10\%$. Nevertheless, the example of the Seychelles warbler illustrates that observations of skewed or changing sex ratios in wild populations should not necessarily be assumed to be an indication of endocrine disruption and that an understanding of the natural history and normal physiology of the species surveyed is essential for the correct interpretation of field data.

Critical developmental stages

Despite the fact that little is understood about the sex-determination process in fish, the limited information available indicates that important species differences exist that should be considered before selection of a species for tests designed to detect endocrine-disrupting effects. For example, the critical period of sex determination in salmonid fish is just a few days around the time of hatch. This is evidenced by the fact that exposure to natural or synthetic estrogens during the few days around the time of hatch will result in the fish all developing as functional females; conversely, exposure to exogenous androgens during this period will result in an all-male population [40], and an inadequate dose of exogenous hormone can result in hermaphrodite fish. This critical window is much longer (weeks) in some species, such as some cyprinid fish, rendering them vulnerable to manipulation of sexual differentiation for a longer period. Also, in contrast to salmonids, exposure of some fish species (e.g., channel catfish) to exogenous androgens during this critical developmental period results in feminization [41]. This paradoxical effect, which was recognized more than a decade ago [41], has hindered the production of all-male channel catfish strains by the aquaculture industry. In addition, some species of fish are hermaphrodites and change sex naturally at some time in their lives [42]. These differences are likely to result in differential sensitivities between species on exposure to endocrine disrupters at early stages of development.

The aquaculture industry has used knowledge of the sex-determination process of salmonid fish to develop monosex (all-female) strains of salmon, providing production advantages by eliminating precocious maturation of males [43]. In addition, this knowledge has been extended further by the development of a rapid test for genetic sex in economically important salmon species [44], allowing the separation of genetic males from genetic females within androgen-treated populations, which are phenotypically all males. In a similar way, to effectively study and understand the effects of endocrine disrupters at critical stages of development, increased knowledge of the factors that affect sex determination in fish and, ideally, the ability to distinguish between sexes at early stages

Table 1. Examples of interspecies differences in sex steroids illustrating qualitative variability within a taxon (e.g., fish) and between taxa (e.g., fish compared to rodents)[a]

	Fish			Mammal
	Salmonid	Tilapia	Ambisexual	Rodent
Primary androgens (in males)	11-KT and T	11-KT and 11-βHT	11-βHT	T, 5-αDHT, 7-αHT[b]
Common function	E.g., development of male secondary sexual characteristics			
Different function	E.g., 11-oxygenated derivatives of testosterone cannot be aromatized and, unlike testosterone, have virtually no negative feedback effect on gonadotropin secretion (aromatization is essential for some effects of androgens at the level of the hypothalamus and central nervous system)			
Primary estrogens (in females)	17β-E	17β-E	17β-E	17β-E, estrone
Common function	E.g., development of female secondary sexual characteristics			
Different function	E.g., stimulation of vitellogenin production by hepatocytes of oviparous vertebrates (c.f. stimulation of uterine growth in mammals)			

[a] T = testosterone; 5-αDHT = 5α-dihydrotestosterone; 7-αHT, 7α-hydroxytestosterone[b]; 11-KT = 11-ketotestosterone; 11-βHT, 11β-hydroxytestosterone; 17β-E = 17β-estradiol.
[b] Found in rats but atypical in mammals as a whole.

of development are required. It is important that research be conducted to further characterize and understand the sex-determination process for any species that may be used in toxicity tests with a view toward producing interpretable data for assessing the effects of endocrine-disrupting compounds.

Need to understand comparative endocrinology

Knowledge of the mechanisms by which chemicals exert their toxic effects in test animals is of critical importance in evaluating risk to humans. This is exemplified by chemicals like d-limonene, which has been shown to induce a specific type of kidney damage in male rats by a mechanism involving α-2-u-globulin, a protein produced in large quantities in the liver of male rats. Extensive research points to the fact that the phenomenon is species and sex specific and is therefore of no significance to humans [45]. In the same way, mechanistic understanding of the action of endocrine disrupters and the confounding effects of interspecies differences in wildlife endocrine systems and function should be considered in the hazard identification process.

It would be misleading to assume that the same hormones exist across species and have the same biological activities (Table 1). For example, in contrast to mammalian species, in male fish the major androgen responsible for the development of male secondary sex characteristics and stimulation of spermatogenesis is 11-ketotestosterone (a nonaromatizable androgen) [42,46]. In addition, there is a wide spectrum among fish extending from those that secrete only 11-ketotestosterone to those that produce only 11β-hydroxytestosterone [42]. Amphibians also exhibit variations in steroid biosynthesis and function since the primary androgens in male urodeles (newts and salamanders) are testosterone, 11β-hydroxytestosterone, and 11β-dihydroxytestosterone whereas the main androgen characteristic of anurans (frogs) is 5α-dihydrotestosterone [47].

Conservation in the main ovarian steroids is extensive because in female amphibians, reptiles, birds, and mammals, these are 17β-estradiol, estrone, and progesterone. Although 17β-estradiol has common functions in females of various vertebrate species, there are also species differences in tissue-specific responses (Table 1). In addition, there is evidence that other hormones may also be important in some species. For example, progesterone is of little importance in fish reproduction, acting only as an intermediate in the synthesis of other derivatives, such as 17α,20β-dihydroxy-4-pregnen-3-one, that play vital roles in final oocyte and sperm maturation before spawning [42].

In addition to qualitative differences in hormones, as illustrated by the preceding examples, there is also considerable species variability in the magnitude of peak plasma levels. For example, peak plasma testosterone levels in female fish can range from >200 ng/ml in rainbow trout (*Oncorhynchus mykiss*) to ~2 ng/ml in bluefish (*Pomatomus saltator*), with corresponding peak 17β-estradiol levels being ~50 and 0.7 ng/ml in the trout and blue fish, respectively [42,48]. In the case of sexually maturing female salmonid fish (e.g., trout), plasma testosterone levels can be an order of magnitude higher than endogenous 17β-estradiol levels at maturity and much higher than the plasma testosterone levels found in males [46,48]. This is unlike the situation in mammals, in which female plasma testosterone levels rarely exceed one-tenth of male values. Such striking interspecies differences may affect sensitivity to endocrine disrupters and may influence test species selection. It should be noted that the above examples of interspecies differences in sex hormones are derived from the few species that have been studied in depth. The reproductive systems of the majority of species remain largely uncharacterized.

The mechanisms by which steroid hormones exert their effects and the extent to which this process varies between species also need to be considered. Steroid hormones exert their influence by interacting with intracellular receptor proteins, which are inducible transcription factors and are highly conserved between vertebrate species [49]. However, there are indications of interspecies differences in receptor affinity, a variable that may also be influenced by temperature and reproductive maturity in some species [50–52]. Recent evidence indicates that two estrogen receptor subtypes exist in rats. These subtypes are thought to exhibit differential expression in various tissues and play an important role in the sensitivity of various tissues to estrogen exposure [53]. It is also possible that several estrogen receptor subtypes exist in birds, reptiles, amphibians, and fish; however, much remains to be determined. Some investigators [54] have noted slight differences in specificity between mammal and fish estrogen receptors, whereas others [55] indicate comparable specificities between fish and human estrogen receptors but different sensitivities.

The extent to which these differences in receptor affinity and specificity influence in vivo responses of different species to endocrine disrupters requires further investigation. Currently, the weight of evidence indicates that the high degree of conservation between receptors of various species generally results in similar binding activities; hence, it is likely that a chemical that binds to rodent uterine estrogen receptors will also bind to fish hepatic estrogen receptors. Therefore, it is probable that lower-tier screening tests using receptors from one species will generally be predictive of receptor binding for many species.

It is well established that ovarian estrogens induce vitellogenin production; however, there is evidence that in some species other hormones may modify this response. For example, in some species of reptiles, growth hormone is an important hormonal requirement for vitellogenin production [56]. However, in amphibians (e.g., *Xenopus*), growth hormone does not appear to play a key role in this process. Nevertheless, even in this species, other hormones appear to modulate estrogen-induced vitellogenesis. Thus, cultured hepatocytes from *Xenopus* lose their ability to respond to estrogen in vitro unless thyroid hormones are added to the culture medium, and tadpoles undergoing metamorphosis will not produce vitellogenin unless exposed to thyroid hormones before exposure to estrogen [57]. Interspecies differences in the hormonal control of vitellogenesis may translate into different sensitivities to endocrine disrupters. For example, in *Xenopus* hepatocyte cultures, the synthetic estrogens diethylstilbestrol (DES) and 17-ethinylestradiol are less potent than 17β-estradiol in inducing vitellogenin mRNA. In contrast, DES and 17β-estradiol are equally potent in inducing this response in the domestic hen [56]. Such interspecies differences in responsiveness should be considered when interpreting the results of in vitro and in vivo assays that rely on the measurement of vitellogenin production in response to estrogen exposure.

It is becoming increasingly clear that there are cell- and promoter-specific actions of steroid hormones that allow the same ligand and the same receptor to give rise to different effects in a variety of tissues [58]. In addition, the importance of various hormone metabolites in controlling and modulating reproductive parameters is becoming increasingly recognized in both humans and other species [59]. Hence, in addition to the fundamental difficulties of extrapolation from in vitro test results to potential in vivo effects, as discussed by Ashby et al. [60], interspecies variations in, for example, receptor-, promoter-, and cell-specific responses add another level of complexity that needs to be considered.

To select appropriate species for standard toxicity tests, appropriately optimized to detect endocrine-disrupting chemicals, it is necessary to have a full understanding of the endocrinology of the test species, including an understanding of the molecular basis of sex determination and availability of markers for genetic sex. Alternatively, further use could be made of monosex strains of fish and other organisms, as illustrated recently by Gimeno et al. [61]. Widespread availability of such organisms could significantly enhance our understanding of the effects of endocrine disrupters in certain wildlife groups. To extrapolate effectively from single-species laboratory tests to diverse wildlife species, it is also essential that our understanding of comparative endocrinology be developed further and that the relative importance of species differences in hormones, hormone metabolites, receptors, etc., be more fully characterized.

OPTIMIZING EXISTING LABORATORY TESTS FOR DETECTING REPRODUCTIVE ENDOCRINE DISRUPTERS

Value of existing wildlife test methods

At present, regulatory hazard assessment guidelines do not include a direct evaluation of specific endocrine parameters in wildlife species. Existing test guidelines do, however, provide for the assessment of ecologically important endpoints (including development, growth, reproduction, and survival) that are relevant to identifying the effects of endocrine disrupters [22]. As illustrated in Figure 1, well-conducted wildlife studies addressing appropriate secondary behavioral and morphological endpoints (level 3) or evaluating individual reproductive effects (level 4) are, in principle, able to encompass primary molecular and biochemical effects caused by endocrine disrupters (level 2).

The availability of a range of regulatory test guidelines, including multigenerational tests, for addressing individual effects in a range of aquatic and terrestrial wildlife species (including birds, fish, and invertebrates) gives further confidence in the value of existing test methods for detecting potential endocrine disrupters [22]. It is recognized, however, that many such wildlife toxicity studies, such as the fish multigeneration tests, are relatively difficult to conduct, are of long duration, and are thus unsuitable for routine screening purposes. To develop a wildlife screening program for endocrine disrupters, both short- and long-term test methods must be optimized to use the resources of government and industry with maximum efficiency.

Mechanistic approach to wildlife effects assessment

It is impossible to test all wildlife species for the potential effects of endocrine disrupters, and ecotoxicologists should develop a mechanistic approach that takes into account the comparative endocrinology of the species of concern. As described previously, an understanding of vertebrate comparative endocrinology and the mode of action of any compound causing reproductive or developmental disorders is needed to assess the ecological significance of exposure for diverse species. A mechanistic understanding will aid ecotoxicologists in extrapolating effects in single-species laboratory tests to potential effects in diverse wildlife species at low exposure levels. Current knowledge indicates that, although not ideal, it is generally appropriate and pragmatic to extrapolate data from tests on rodents to other mammalian wildlife species and, to some extent, to other nonmammalian wildlife species. However, further research may be required to determine whether mammals that exhibit unusual reproductive traits, such as delayed implantation (e.g., mustellids, pinnipeds), require special consideration. In addition, it should be recognized that relatively little is understood about potential interspecies sensitivities of invertebrates. For example, although daphnids are widely used for reproductive effects assessment of new and existing chemicals, relatively little is known about the reproductive endocrinology of daphnids [62], and organisms such as *Daphnia magna* appear to be relatively insensitive to compounds such as DES compared with vertebrate species [63]. This apparent insensitivity of daphnids is likely related to their asexual mode of reproduction but should not undermine the overall importance of daphnids in ecological hazard assessment since they occupy an essential niche in aquatic ecosystems. However, further research is needed to define the reproductive endocrinology of daphnids and other regulatory invertebrate test spe-

- **Ecological field studies**
  - Collate published information on the population status of wildlife species potentially exposed to EDCs
  - Identify sentinel species for ecosystem monitoring (eg molluscs, fish, etc.)
  - Evaluate baseline patterns in reproductive endocrinology in sentinel species, taking into account seasonal cycles and critical exposure periods
- **Laboratory studies**
  - Understand the comparative endocrinology of candidate test species
  - Develop *in vivo* assays for detecting EDCs in key wildlife species; use these 'gold standard' assays to support further utilisation of *in vitro* assays
  - Develop the links between short-term assays and long term effects on survival, development and reproduction
  - Quantify the links between ecologically important *in vivo* effects and biomarkers* of exposure (e.g. vitellogenin production)
- **Linkage studies (Field<------> Laboratory)**
  - Develop and validate biomarkers of exposure for in situ monitoring; understand their ecological significance
  - Develop markers of genetic sex for application in field and laboratory studies.

Fig. 3. Summary of research priorities for hazard assessment of endocrine-disrupting compounds (EDCs) in wildlife. The term "biomarker" is considered to incorporate biochemical, histological, and physiological indicators of exposure to xenobiotic chemicals at the suborganismal or organismal level (adapted from Huggett et al. [68].

cies so that the results from studies can be better extrapolated to invertebrate populations in natural ecosystems. Such fundamental research should also be applied to define the mechanistic relationships between fish species currently used for testing and amphibians and other oviparous vertebrate species [10].

LINKING LABORATORY TESTS WITH FIELD OBSERVATIONS AND MONITORING STUDIES

When addressing the research needs for developing screening tests for endocrine disrupters in wildlife, it is important to recall that it was field studies conducted in Europe and North America that initially drew attention to this important question [5]. To address, for instance, whether the observations of reproductive abnormalities in fish [19,23], reptiles [8], and birds [24] are evidence of a widespread problem or a localized phenomenon, it is essential that refined field studies be undertaken using complementary biological and chemical techniques. In this rapidly developing area of ecotoxicology, it is important that screening tests of endocrine disrupters be interpreted in light of high-quality field data. This is especially true when there is a need to use in situ biological monitoring techniques to investigate potential endocrine effects in aquatic organisms exposed to effluent discharges and to use the same techniques for complementary laboratory toxicity identification evaluations (TIEs) [64].

Currently, an important opportunity exists to undertake wildlife research toward codeveloping techniques for evaluating endocrine disrupters in laboratory tests and field monitoring programs. In particular, the proposed laboratory studies aimed at defining the relationships between the primary and secondary responses to exposure to endocrine disrupters (Fig. 1) will provide important validation studies that will assist in the application of suitable biomarkers (defined as molecular, biochemical, and cellular effect measurements) for use in field monitoring studies. This work should be greatly assisted by the use of a mechanistic approach so that results from laboratory test species can be related to field monitoring species, as exemplified by the recent work on the vitellogenin assay for a variety of cyprinid species [65]. In this regard, it is important that controlled laboratory studies be conducted to assess the relationship between health effects and increased vitellogenin production by fish exposed to exogenous estrogenic substances while parallel and complementary field monitoring studies are conducted. By linking field and laboratory studies, the ecological significance of increased plasma vitellogenin in a wild population can be more effectively assessed. Another example of the ability of well-conducted laboratory studies to facilitate the analysis of field monitoring can be foreseen in the area of sex determination. An understanding of the molecular basis of sex determination in wildlife species, such as fish, would profoundly aid interpretation of field data, particularly when attempting to evaluate the significance and cause of hermaphrodism in a population. In addition, natural levels of hermaphrodism in control fish populations need to be determined by field surveys before the significance of this condition can be assessed in other populations. Hence, it is vital to link appropriate laboratory and field studies to encompass the ecological dimension in this debate.

RESEARCH NEEDS FOR ENDOCRINE DISRUPTERS AND WILDLIFE

Because the reproductive success of a species is the key to its ecological viability, improved understanding of the significance of endocrine disrupters for the reproductive health of wildlife is urgently needed. It is therefore recommended that (1) internationally compatible databases be established to collate information on the status of ecosystems purported to be at risk from endocrine disrupters and other chemical contaminants, (2) further research be undertaken to examine population status and trends in species apparently at greatest risk, and (3) investment be made into basic research that addresses the reproductive consequences of exposure to endocrine disrupters in wildlife.

To develop an effective hazard identification strategy for endocrine disrupters, a number of research objectives need to be addressed (Fig. 3). Any indications that wildlife populations are experiencing stress and signs of reproductive impairment

should elicit concern and further study to determine the factors responsible. Once identified, these factors should then be tackled as a priority. From a European perspective, the present uncertainty regarding the ecological relevance of endocrine-disrupting compounds may provide a valuable opportunity for governmental environmental agencies to coordinate the establishment of an appropriate ecological database. The database established by the U.S. Department of the Interior is an impressive example of such a database [10].

It is important that appropriate sentinel species be identified. Ideally these should have the following characteristics: widespread distribution, a well-characterized endocrine system that is representative of the taxa, ease of maintenance in the laboratory, ease of sampling in the field, and sensitivity to endocrine disrupters. In addition, it is vital that important assays for wildlife assessment be refined, standardized, and fully validated before being incorporated into any hazard identification scheme for endocrine disrupters. Such validation procedures must include a requirement that the results of novel tests or biomarkers are fully interpretable in terms of predictiveness for adverse health effects in whole organisms. It is also essential that present testing strategies be assessed to determine the extent to which they can detect compounds acting through an endocrine-disrupting mechanism. Potential refinements to existing tests to improve their sensitivity for this purpose should be fully investigated and validated before incorporation into existing test guidelines.

From an ecological perspective, it is also important that our understanding of vertebrate and invertebrate comparative endocrinology be further improved and that the normal seasonal cycles of sentinel wildlife species, including natural variability in reproductive parameters (e.g., hormones and receptors), be determined. In addition, there is a need to elucidate the molecular and cellular mechanisms by which endocrine disrupters act because this information can then be used to predict comparative effects across species and potential for widespread effects following exposure. Further understanding of the sex-determination process in species currently being used as wildlife sentinels (e.g., cyprinids) in both laboratory and field studies is also needed. This information is critical for interpreting results from studies assessing effects on sex determination and development in fish.

Finally, it is essential that attempts to evalulate wildlife population health and exposure to endocrine disrupters, in both laboratory and field studies, be coordinated on an international level to ensure maximum use of finite resources, to prevent duplication of effort, and to ensure international harmonization of data interpretation and any subsequent regulatory responses. Premature implementation of testing and assessment methods for endocrine disrupters without first completing the essential research required and without appropriate consideration of the points discussed in this report may result in assessment schemes that placate public opinion but neither alleviate the major factors impacting wildlife populations nor effectively protect diverse species with distinctive endocrine systems.

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