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Topic 4.1

Historical perspective on endocrine disruption in wildlife*

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Abstract: Endocrine disruption (ED) as a named field of research has been very active for over 10 years, but effects in wildlife that would now be labeled as ED have been studied since the 1940s. This paper briefly surveys the progress in wildlife studies that has been made to date and draws out the major themes and issues that have been identified. In particular, it discusses information concerning causative substances, modes of action, ubiquity of effects across taxa, individual- and population-level impacts, and the importance of low-dose and mixture effects. The main conclusion is that while most wildlife taxa are showing some ED effects at some locations, good evidence for population-level impacts is still limited to a few groups. In order to improve both the interpretation of field observations and the way in which environmental risk assessments are conducted, we need to develop an enhanced ability to predict effects on populations and communities from a knowledge of effects on individuals.

BEFORE "ENDOCRINE DISRUPTION"

It is a common misconception that the study of endocrine disruption (ED) began with the famous meeting at the Wingspread Conference Center, Racine, Wisconsin in July 1991 organized by Theo Colborn and coworkers [1]. Of course, there is no doubt that the current interest in this subject as a named research field dates from that time. For example, using the search term "endocrine disrup*" with the Science Citation Index (ISI Web of Science) in May 2002 brought up 1346 research article references, the first of which is Colborn et al. (1993) [2]. In fact, the first published mention of ED seems to be in Colborn and Clement (1992) [1]. Figure 1 shows how the field has exploded since then in terms of published papers, although it should be borne in mind that many relevant articles do not contain endocrine disruption in their titles, abstracts, or keywords. The true total of papers that have addressed the subject since 1993 is in excess of 2000 [26]. On the other hand, some of these relate to possible endocrine disruption in humans and mammalian models, and it is much harder to estimate the research activity on the wildlife side. Since 1981, for example, there have been 376 research papers alone that deal with tributyltin (TBT) and wildlife, and another 288 concerning alkylphenols and wildlife, so the total for all categories of endocrine active substances (EASs) is probably over 800. Of course, one of the almost unique features of this field of study, as exemplified by Colborn et al.'s first paper [2], is the close collaboration between wildlife scientists and clinicians or medical scientists, so perhaps we do not need to strive too hard to tease humans and other organisms apart.

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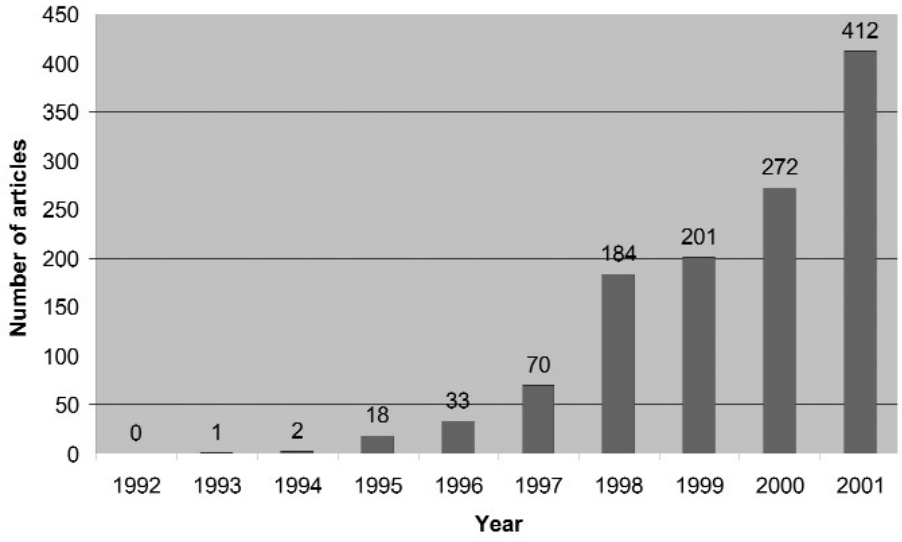

Fig. 1 Annual number of published scientific articles that refer to endocrine disruption. Note that articles which do not use this phrase are not included.

In reality, the study of ED has been in progress for much more than 10 years, although the full range of early work is not represented in Colborn and Clement [1]. Perhaps surprisingly, this book only contained 4 chapters (out of a total of 21) on evidence for ED in wildlife—Chap. 6 by W. P. Davis and S. A. Bortone on the effects of pulp-mill effluent on the sexuality of fish; Chap. 7 by J. F. Leatherland on thyroid abnormalities in Great Lakes salmon; Chap. 8 by G. A. Fox on abnormal sexual development in North American birds, but including some mention of TBT-related imposex in mollusks, and effects of pulp-mill effluents on fish; and Chap. 9 by P. J. H. Reijnders and S. M. J. M. Brasseur on hormonal disorders in marine organisms including mammals. Theo Colborn's major achievement in 1991 was to pull together information from very disparate fields of study (including both wildlife and humans) and come up with a new paradigm of toxicant action, but research in fields that would now be labeled as ED had in fact been published since the 1940s and 1950s. For example, work in the 1940s [3,4] revealed ED effects in livestock caused by natural and synthetic substances, respectively. Furthermore, synthetic substances with endocrine action had been known since the 1930s (e.g., estrogenic activity of diethylstilbestrol [5]). Even earlier, in the 1920s, it had been discovered that sexual condition could be induced in immature female rats by injecting small amounts of what was then known as ovarian follicular hormone from pigs [6], so the incredible potency of hormonally active substances is not a new idea.

As we now know, the new concept in 1991 was the attempt to explain many pathologies and other abnormalities in wildlife (and humans) in terms of endocrine interference through hormone mimicry and other mechanisms. Up until that time, the idea that synthetic substances with only slight or no resemblance to hormones or their antagonists, could be causing widespread biological effects in the natural world through interference with endocrine systems would have been dismissed as fanciful by all but a few. Furthermore, the effects on wildlife of discharges of naturally occurring hormonally active substances (e.g., phytoestrogens and androgens in pulp-mill effluent) had only been studied to a limited extent at that date.

Endocrine disruption is sometimes dismissed as an issue of primarily academic interest (or even self-interest), with little if any relevance for real ecosystems. This follows in a long tradition employed by critics with various axes to grind, who either deliberately or accidentally overlook the abundance or significance of evidence for particular phenomena. The present volume, for example, brings to-

gether an overwhelming body of knowledge which shows not only that ED in some wildlife is a widespread reality, but that it can cause population- or community-level damage given the right combination of conditions. There is certainly honest debate about the extent of such damage, but the quantity and quality of available data now make it clear that ED is a common mechanism whereby a range of pollutants is able to cause effects in wildlife. In earlier eras, similar "critical masses" of knowledge had to be assembled for toxicological phenomena caused, for example, by carcinogens, teratogens, and persistent organochlorine (OC) pesticides, before critics were forced to retreat. The best-known example of such an assembly of knowledge is probably Rachel Carson's final book *Silent Spring*, published in 1962, which blew the whistle about the impacts on wildlife being caused by OCs [7]. Interestingly, Carson drew attention to the ability of certain OCs to interfere with reproduction, although at that time the endocrine-disrupting mechanisms were unclear. Partly because she also attacked our unsustainable exploitation of nature, and partly because *Silent Spring* was written as a (highly literate) polemic rather than as a scientific treatise, Carson was heavily criticized by a variety of industrial interests, but time has of course proved her largely correct, both about OCs and about the need for sustainability.

In a real sense, these earlier discoveries prepared the ground for our study of ED. Crucially, they accustomed the world of ecotoxicology to the idea that certain substances in extremely small doses could have far-reaching biological effects which were completely unrelated to their acute toxicity. It is interesting to note, however, that while regulation of certain chemicals began to incorporate rodent-based tests for carcinogenic and teratogenic effects (essentially to protect humans), protection of wildlife against such substances was treated as a lower priority. Thus, even now, environmental risk assessment of most chemicals is based on acute toxicity data to which is applied some sort of empirical "safety factor", and it is doubtful whether such notorious pollutants as TBT would be caught even by the more sophisticated testing screens which are now applied to pesticides and biocides. It will probably be a few years yet before environmental risk assessment of chemicals for effects caused by ED becomes routine (see Topic 4.12 for information on the development of fish-based ED tests [8]).

DEVELOPMENTS SINCE 1991

Since 1991, several additional abnormalities in wildlife have been recognized as stemming from ED. These include the feminization of fish by natural and synthetic estrogens in sewage effluent (see Topics 4.3 and 4.5); abnormal interrenal function leading to weakening of the stress response in fish exposed to a range of contaminants [9]; and altered sex determination and differentiation in alligators and other reptiles which in some contaminated locations has been associated with population declines (Topic 4.7). There is also some evidence that limb deformities in amphibians may be caused by a form of ED related to environmental retinoids (e.g., Topic 4.6). Furthermore, laboratory studies have shown that amphibians can be feminized or demasculinized by EASs [10,11], although the available evidence suggests that the widespread declines in amphibian populations are probably related in complex ways to global warming rather than chemical pollution [12].

Much more information has become available since 1991 on the effects of organotins in mollusks which first appeared in about 1975 soon after TBT was introduced in antifouling paints [13]. TBT-induced masculinization in both bivalves and gastropods is now definitely established as a form of endocrine disruption which has led to invertebrate population and even community declines (e.g., Topic 4.2 [13,14]). On the other hand, although more information has also been accumulated on ED in birds (e.g., Topic 4.8 [15]), the data on effects in the field are still rather sparse, and it is even disputed whether ED occurs at all in wild birds [16]. DDE-induced eggshell thinning in raptors is often cited as an example of ED which had been observed as early as the 1960s [17], but the true mechanism has been elusive. The effect is probably a direct inhibition of prostaglandin synthesis in the shell gland mucosa, which in turn interferes with calcium metabolism [18], but it is a moot, though trivial, point whether this qualifies as an example of ED. Finally, it is perhaps surprising that more is not known about ED in

mammalian wildlife given the extensive literature on experimentally induced ED in rodents, mustelids, and primates (several authors—present volume; [19]). The data on ED in some wild aquatic mammals are reasonably convincing (Topic 4.4), although experimental results are understandably sparse, but information from terrestrial mammals is still restricted to a few equivocal studies [20]. Whether this is due to genuine differences from aquatic mammals in exposure or susceptibility, or simply to an absence of suitable field investigations, is not clear.

In recent years, we have seen a plethora of books on ED and wildlife, none of which (in my opinion) quite match up to *Silent Spring* in terms of readability or impact. Nine of these were ably reviewed by David Peakall shortly before his untimely death [21]. They range from popular books in the *Silent Spring* tradition [22,23], to highly detailed scientific works that are similar in scope to the present volume [24]. Probably the most useful documents not reviewed by Peakall are Kime [25] which exhaustively covers ED in fish, and Damstra et al. [26] which reviews the effects of ED in both humans and wildlife for the International Programme on Chemical Safety. There are, of course, several others mainly concerned with possible ED in humans [27]. Finally, there are numerous web sites dedicated to ED in both wildlife and humans, including these useful ones: <http://endocrine.ei.jrc.it/index.html> maintained by the European Union, <http://www.epa.gov/endocrine> by the United States Environmental Protection Agency, <http://www.nihs.go.jp/hse/environ/endocrine.html> by the Japanese Institute of Health Sciences, and sites maintained by nongovernmental bodies such as Tulane University <http://www.som.tulane.edu/ecme/eehome/>.

MAJOR FEATURES OF ENDOCRINE DISRUPTION IN WILDLIFE

It is not the purpose of this article to review in detail the effects of EASs in wildlife—that job is done by other chapters in the present volume. Instead, with the benefit of the many years of research referred to above, the intention is to draw out some broad conclusions about how the field has progressed to date.

Causative substances

The list of potential EASs is now very long and diverse. For example, the European Union has published a candidate EAS list consisting of 553 man-made substances and 9 synthetic or natural hormones, which although very tentative (as it is based on patchy data and nonstandard testing methods), at least gives a crude picture of the possible scale of the problem [28]. This section focuses on those substances strongly suspected of causing effects in wildlife (as opposed to those that have been identified experimentally as EASs).

- Many examples of ED that have been reported in feral wildlife concern estrogens and their mimics causing receptor-mediated effects. For example, in the case of vitellogenesis and ovotestis in male fish, there is a strong association with estrogenic substances in treated sewage effluent [29,30]. Of these estrogens, natural and synthetic hormones appear to comprise the major part, although synthetic estrogen-mimics such as nonylphenol also contribute some effect. On the other hand, androgens in sewage do not appear to be a major issue for aquatic life [31], although natural substances in pulp-mill effluent can have androgenic effects in fish [32]. Pulp-mill effluent is actually a complex mixture of phytoestrogen derivatives and natural phenolics [33,34], both of which can cause a range of endocrine disturbances, not just at the receptor level, but also on various parts of the pituitary-gonadal axis [35].
- A ubiquitous group of contaminants, the polycyclic aromatic hydrocarbons (PAHs), is suspected of having a range of weak ED effects (depending on structure), including estrogenicity caused by agonistic action [36], antiestrogenicity via Ah-R and ER binding [37,38], androgenicity and antiandrogenicity—both AR-mediated [39]. PAHs do not biomagnify in vertebrates due to rapid metabolism, so they are unlikely to cause effects at a distance, but field studies in highly PAH-con-

taminated areas such as Puget Sound have revealed a range of possible ED effects in flatfish (e.g., delayed gonadal development, reduced reproductive success, and precocious female development) which have been attributed inter alia to PAHs [40–42]. It is difficult to attribute causality with precision, however, due to the frequent co-occurrence of PAHs in Puget Sound with various OCs.

- Of the other effects in wildlife which have been proven or strongly suspected to be caused by ED, the overwhelming majority are linked to the persistent organochlorines (OCs), especially the DDT, dioxin, and PCB families. Various isomers and congeners in these groups have been shown in the laboratory to affect steroid hormone levels or act agonistically/antagonistically at steroid and/or aryl hydrocarbon (Ah) receptors, and a host of field studies have implicated OCs in reproductive effects in various wildlife, from fish to mammals. One of the most well-studied phenomena concerns the impact of organochlorine contaminants on reproductive success of alligators in Florida (Topic 4.7 [43]), which have experienced altered sex hormone titres and reproductive tract anatomy that have in turn probably been the cause of population declines in some locations. Key features of many OCs are their persistence, long-distance transport, bioaccumulation and biomagnification. These properties imply that OC-related ED may occur in top-predators at locations remote from human activity, and this indeed could be happening in some wildlife groups ranging from swordfish to polar bears [44,45].
- Turning to synthetic nonestrogens/androgens, the major example identified to date is probably TBT, which interferes with testosterone metabolism in mollusks, causing imposition of male characteristics in females and reduced reproductive success [14,46]. TBT, although a biocide used in antifouling paints, was not designed to be an EAS. On the other hand, a range of substances such as ecdysteroid agonists and antagonists, and juvenile hormone analogs, have been deliberately designed as pesticides to control insects by ED, although there is little evidence that nontarget arthropods have been damaged by these pesticides under field conditions [47].
- Finally, it has been established that interrenal dysfunction can be caused in wild fish and other vertebrates by chronic exposure to a range of organic and inorganic pollutants, including heavy metals, PAHs, and PCBs (Topic 4.10 [9,48]). These effects have mainly been studied in North America, but in essence involve an impairment of the secretion of corticosteroid hormones such as cortisol. The affected organisms consequently develop a reduced ability to respond to stress, which in principle can cause a variety of deleterious outcomes. These effects deserve wider study because they are likely to result from virtually any kind of chronic pollutant exposure.

Modes of action

It will be apparent from the cases cited above that a wide range of modes of action has been found or strongly suspected to occur in wildlife. Of the receptor-mediated mechanisms, there is abundant evidence for estrogenic effects, mainly in connection with sewage effluent, and support also exists for activity in antiestrogenic, androgenic, and antiandrogenic pathways at some locations. Unsurprisingly, these result in various interferences with sex determination, sexual maturation, and/or successful reproduction. Agonistic and antagonistic actions at the ecdysteroid receptor also undoubtedly occur in insects in response to certain insecticides and phytochemicals [47,49], causing interference with molting, but it is unclear if other arthropods are also affected. Other suggested mechanisms (e.g., agonistic or antagonistic action by exogenous substances at retinoid receptors in amphibians, causing abnormal limb development—Topic 4.6) have not yet been widely established in the field. However, nonreceptor-mediated mechanisms are widespread, including interference with the synthesis, metabolic breakdown or excretion of hormones in a range of organs, and blockage of the controlling links between the pituitary and the hormone-synthesizing tissues. There are even examples of so-called "cross-talk" from one hormone system to another, such as the suspected interference with the thyroid system of fish (thyroxine build-up) due to pollutant-induced exhaustion of the cortisol response [50]. The reasons for other thy-

roid abnormalities, for example in Great Lakes salmonids, may not, however, be directly linked to contaminants [51,52]. Finally, there is evidence that larval exposure to some EASs can lead to heightened sensitivity to these substances in the resulting adults, presumably as a result of increased synthesis of hormone receptors [53], although this has not yet been proven to occur in the field. All these complex modes of action and interaction mean that the work of uncovering causative substances, or of predicting effects, can be very complicated. Indeed, one can only be confident of having arrived at the right explanation for abnormalities seen in the field if they can be satisfactorily replicated under laboratory conditions and then abolished or minimized by regulation of the suspect substance(s).

Ubiquity of effects across taxa

It is becoming clear that endocrine disruption can occur in a very wide range of animals. As well as having been observed (either in the field or the laboratory) in every class of vertebrate [24,54], it has also been recorded in insects, crustaceans, and mollusks [47]. It probably also occurs in echinoderms. By extension, it can therefore be expected in all animals which use some form of hormone signalling. This is not to say that the situation is well-studied in all the taxa referred to above. For example, although we now know rather a lot about ED in fish, our knowledge about other taxa tends to be sketchy. This particularly applies to most invertebrate phyla whose endocrine systems are poorly understood by comparison with vertebrates. However, vertebrate endocrinology is sufficiently similar across the phylum that it becomes feasible to use certain wildlife species as sentinels for effects in humans, always bearing in mind that exposure to EASs may differ greatly. It is noteworthy that ED seems to be particularly associated with aquatic wildlife [55], and this may be a consequence of the high pollutant exposure which water-breathers and their predators receive.

Individual- vs. population-level effects

The overwhelming majority of unequivocal ED effects seen in the field have been at the level of the individual. These range from subtle changes in biochemistry (e.g., VTG induction; altered hormone titres), through cellular changes (e.g., ovotestis) and gross morphological effects (e.g., imposition of secondary sexual characteristics; delayed gonadal development), to impacts on whole-body functions such as reproductive success (e.g., reduced egg-laying in TBT-exposed gastropods). On the other hand, there are few clear examples of population declines which have resulted from these processes, although the cases of TBT in mollusks and OCs in alligators, which have both been accompanied by reduced abundances in some locations, are the exceptions which prove that such effects are possible. DDE also caused population declines in some raptors due to eggshell-thinning, although it is still debated whether this is an example of ED. There are many other examples of population effects which have been tentatively attributed to ED (e.g., epizootics and population declines in some marine mammals; global declines in amphibians; reduced raptor populations; declines in Great Lakes salmonids), but causal relationships in these cases remain unclear [54]. This is, no doubt, partly due to the difficulty of proving cause and effect in species which are not amenable to experimentation, but it must also be recognized that not all impacts on individuals are translated through to the population level. A graphic illustration of this is provided by the phenomena of ovotestis and vitellogenesis in UK roach (*Rutilus rutilus*) populations where up to 100 % of males have been affected in severely estrogen-contaminated rivers without loss of populations [56]. Ovotestis in wild *R. rutilus* is accompanied by reduced gamete production [57] and probably by reduced reproductive success, but apparently not enough completely to overcome mitigating processes such as density dependence. This is not to say that population-level effects must necessarily be proven before ED is taken seriously, but they should at least be plausible in the light of the biology of the species in question.

Importance of low-dose effects and mixtures

Traditional ecotoxicology and environmental risk assessment are founded on the assumption that, below a certain dose (the threshold), no effects can occur due to the action of repair and excretory mechanisms, etc. However, as pointed out by Sheehan [58], if a dosed contaminant mimics an endogenous agonist, the threshold for effect will already have been exceeded at zero dose. This is supported by an analysis of 31 experimental mammalian ED datasets, 26 of which demonstrate modified Michaelis–Menten kinetics that have no threshold [59]. Similar dose–response relationships have been seen in temperature-dependent sex determination of estradiol-dosed turtle embryos [60], so it is not unreasonable to expect that such effects are occurring in wildlife populations. The situation is further complicated by the fact that some EAS dose–response curves are not monotonic. For example, it has been shown [61,62] that low estradiol doses in developing mouse embryos stimulate the growth of the prostate gland, while higher doses prevent it getting so large. These effects have not been shown to occur in free-living wildlife because it is usually impossible to have an adequate knowledge of dose under these conditions. However, the major implication is that environmental risk assessments which are often based on testing high concentrations in acute tests may tend to underestimate the effects of the low concentrations which usually occur in the field (Topic 4.9). Risk assessment becomes even more problematical when one considers that agonists or antagonists and their mimics can all act via the same receptor, so receptor-mediated ED in complex environmental mixtures is likely not to be predictable on the basis of the known concentration of a single EAS. Such EAS interactions have been shown in vivo to be less-than-additive, additive, or synergistic, depending on the organism, endpoint, and mixture [63–68], but much more work needs to be done to understand this complex field. At present, it is clear that the discovery of low dose and mixture effects of EASs will force a fundamental reappraisal of environmental risk assessment procedures for EASs.

CONCLUSIONS

The enormous amount of activity in the field of ED in wildlife over the last 10 years has born much fruit. It is now clear that almost all major wildlife groups are experiencing ED at some contaminated sites. Furthermore, such effects are being caused by a wider range of natural and synthetic substances than was anticipated in 1991, and by a wider range of mechanisms. It now seems likely that ED at many sites is caused by complex mixtures of substances, often acting at low concentrations which could not have been predicted on the basis of traditional toxicity tests using high concentrations of test substances. This has major implications for the environmental risk assessment of EASs. In a few cases, we now have convincing evidence that ED can lead to population declines and even disturbances at the community level, but our ability to predict such higher-order effects is still weak. This is a reflection of our imperfect knowledge about ecosystem structure and function, and is a substantial challenge for the future if we are to improve our interpretation of field observations.

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