Effects of endocrine disrupters in wild birds and mammals

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Abstract

Effects of endocrine disrupting compounds (EDCs) have been seen in wildlife. Five case studies are presented and discussed in relation to a framework to determine the strength of the evidence that an EDC is responsible for the effect, and that the effect is endocrine-mediated. The five cases are eggshell thinning in birds related to DDE exposure, chick edema disease related to PCDD/F exposure, Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) in birds related to dioxin-like compounds, reproductive dysfunction in mustelids and in Baltic Sea seals related to PCBs and dioxin-like compounds. In general, strong associations are found between the effects and the exposure to the particular EDCs. However, associating the effect to an endocrine-mediated mechanism has been shown to be more difficult.

Introduction

An endocrine disrupter is defined as an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations (1). The best studied examples of endocrine disrupters (EDC) associated with effects in wildlife are dichlorodiphenyldichloroethylene (DDT) and its metabolite p,p'-dichlorodiphenyltrichloroethylene (\(p,p'\)-DDE), polychlorinated biphenyls (PCB) and polychlorinated dibenzo-p-dioxins and furans (PCDD/F). In this presentation, I would like to make a distinction between effects of endocrine disrupting compounds and endocrine disruption. In laboratory studies, administration of an EDC to laboratory rats may disrupt or effect hormones or other aspects of an endocrine system directly or indirectly. If the mechanism of endocrine disruption is known and understood for this compound, then we can say that this is both an effect of an endocrine disrupter and that it is causing endocrine disruption. It should be understood though that many endocrine disrupters can
also cause effects that are not necessarily mediated by endocrine systems and thus the effect is not a form of endocrine disruption. Also, there are a number of stressors that can cause effects in wildlife. For example, disease, starvation, reproductive phase etc. can cause similar effects as those seen from exposure to endocrine disrupters. Thus, the effects seen in wildlife could be evidence of endocrine disruption, but might also be caused by other stressors than endocrine disrupters. There is also the case where endocrine disruption is suspected to be the cause of an effect, such as reduced reproduction, but the data to link a specific EDC to the effect are not sufficient to rule out confounding factors.

A major problem with linking a specific endocrine disruption effect to a particular EDC is that environmental exposure is complex. Wildlife are exposed to a cocktail of contaminants and the exposure to these varies over their lifetime. This makes it very difficult to establish a causal link between one particular EDC or EDC group and a specific effect seen. Thus, it is usually only possible to study whether or not there is a statistical association between an effect and an EDC.

By using laboratory studies, semi-field studies and field experiments in addition to correlative field studies, it may however be possible to build a weight-of-evidence case that is much stronger. In a recent WHO report, a framework for assessing the relationship between EDC exposure and endocrine effects has been proposed (2). Within the framework, a clear hypothesis needs to be made containing two distinct parts. First, the effect must be linked to an EDC that acts on the individual or population. Second, exposure to the EDC must result in endocrine-mediated events that lead to the effect. The scientific evidence is then evaluated using five criteria: temporality, strength of association, consistency of observations, biological plausibility of the effect and evidence of recovery when the EDC exposure is reduced.

Temporality determines whether exposure to the EDC being studied preceded the observed effect. The strength of association looks at the incidence of an effect and the various variables that could explain this and whether or not exposure to an EDC could be involved. The consistency of observations is based on reviewing the literature to see how often similar or dissimilar conclusions are found, including if results come from many geographical areas, if different species would be expected to react in a similar manner and at similar exposure levels. Biological plausibility includes evaluating all research that helps to determine the mechanism of endocrine disrupter action for the EDC. Evidence of recovery studies whether the effect is reversible when exposure decreases. Using the hypothesis and these five criteria, an overall strength of evidence evaluation can be made as to whether or not there is a relationship between the effect and exposure to an EDC and whether or not this effect is due to endocrine-mediated mechanisms. With this framework in mind, several case studies of the effects of endocrine disrupters in wildlife will be discussed.
Case studies

**Birds**

*Eggshell thinning*

In the 1960s and 1970s, population declines were seen globally for several birds of prey and in colonial water birds (3). The peregrine falcon (*Falco peregrinus*) was almost driven to extinction. The cause was found to be eggshell thinning, which led to eggs that cracked or broke under the weight of the adults during incubation (4,5). This in turn led to reproductive failure. The eggshell thinning was suspected to be due to the widespread agricultural spraying of DDT. To test the hypothesis that DDT might be the cause of the eggshell thinning, British scientists studied archived eggs from museums and private collections. They found measurable reductions in eggshell thickness, for example, in sparrow hawk (*Accipiter nisus*) starting abruptly in 1947 when DDT was introduced into agricultural use (4,6). Measurements of DDT levels in the field showed strong correlations between increasing egg concentrations of a DDT metabolite, *p,p*-DDE and decreased shell thickness, for example in peregrine falcons (reviewed in Peakall et al. (7)). Laboratory studies where kestrels (*Falco sparverius*) were fed *p,p*-DDE showed a clear dose-response effect between increasing DDE concentrations in the eggs and decreasing egg-shell thickness (8).

The laboratory data were compared to results from studies of wild kestrels and the same relationship was seen with similar DDE concentrations causing similar degree of eggshell thinning in both laboratory and wild kestrels (8). Thus the causative agent of eggshell thinning was concluded to be *p,p*-DDE. When DDT was banned in many countries, eggshell thickness began to increase in affected bird species. Later mechanistic studies found that DDE affects the shell gland, leading to altered prostaglandin synthesis in the mucosa gland (9) and this is suspected to be the mechanism. However, different species show differing effects on eggshells so the link to endocrine disruption is not completely clear.

*Chick edema disease*

In the 1950s, millions of domestic chickens died mysteriously in the United States. The symptoms they exhibited included reduced weight gains, droopiness, ruffled feathers, edema in the heart sac and abdomen and death, and the disease was dubbed “chick edema disease”. An unknown contaminant in the fat used in making the chicken feed was implicated as the cause (10). Eventually, the causative agent was found to be 1,2,3,7,8,9-hexachlorinated dibenzo-*p*-dioxin (HxCDD) (11). Extracts of the feed injected into chicken eggs caused decreased hatching, embryo deformities such as beak and eye defects, edema and decreased body weight. Laboratory studies found similar effects with other PCDD/Fs and with several dioxin-like PCBs (coplanar PCBs)(reviewed in Gilberston et al. (12)). Domestic chickens were found to be extremely sensitive to the effects of dioxin-like compounds. A recent outbreak of chick edema disease was seen in Belgium in January 1999. High PCB levels were found in the feed and illegal
dumping of PCB oil into the fats used in making animal feed was suspected to be the cause (13). The PCB oil contained about 1 g of TCDD equivalents (TEQ) from PCDFs and 2 g TEQ from coplanar PCBs. The link to endocrine disruption is however not clear.

**Great Lakes Embryo Mortality, Edema and Deformities Syndrome (GLEMEDS)**

When DDT levels declined in colonial piscivorous birds from the Great Lakes area, eggshell thinning also declined. However, reproductive impairment still continued. As more eggs made it to hatch, scientists noticed high levels of embryo and chick mortality and that many chicks exhibited deformities such as club feet and crossed bills, cardiac edema, decreased body weight and skeletal malformations (12,14,15). These effects had not been seen previously because the eggs had been crushed and the embryos not able to develop. The species primarily affected were herring gulls (*Larus argentatus*), double-crested cormorants (*Phalacrocorax auritus*), Forster’s (*Sterna forsteri*) and Caspian terns (*Sterna caspia*). These abnormalities were defined as the Great Lakes embryo mortality, edema and deformities syndrome (GLEMEDS) (12,16).

These new effects indicated that another causative agent than DDT was responsible. The similarities were so strong to those of chick edema disease that scientists suspected dioxin-like compounds to be the cause (12,17). Several studies found strong correlations between the concentrations of dioxin-like compounds expressed as TCDD equivalents (TEQ) and embryo mortality in double-crested cormorants (18) and deformities in double-crested cormorant and Caspian tern chicks (14,19). Researchers also performed experiments both in the laboratory and the field to test this hypothesis. For example, they made extracts of colonial bird eggs and injected these into chicken eggs and found that this induced chick edema disease. Using all the data that they had collected, they then applied similar criteria as the WHO framework, using the term ecoepidemiology, and concluded that the effects were strongly associated and most probably due to exposure to dioxin-like compounds (12,14,20-22). Declines in the levels of PCBs and PCDD/Fs in the Great Lakes has been followed by improvements in reproduction and reduction in the symptoms of GLEMEDS (12,23). The mechanistic link to endocrine disruption has not been clearly established, however.

**Mammals**

**Reproductive dysfunction in mustelids**

In the 1960s, reproductive failure was seen in ranched mink (*Mustela vison*) fed fish from the Great Lakes (24). These fish were found to contain high concentrations of a range of organochlorine compounds, many of them EDCs. Population declines were also seen for wild mink and otter (*Lutra canadensis*) in North America, particularly around the Great Lakes region, and in wild otter (*Lutra lutra*) in Europe. Experimental studies showed that mink fed a diet of Great
Lakes salmonids had negative reproductive outcomes (25,26). In another study, mink were fed environmentally relevant doses of a technical PCB (Clophen A50), and several different fractions of this PCB mixture, including the fraction containing the dioxin-like PCBs. Increased rates of fetal deaths, abnormalities, decreased kit survival and decreased kit growth were seen and the effects were associated with the dioxin-like PCBs (27). Similarly, reproductive impairment in Great Lakes mink was linked to exposure to dioxin-like PCBs and PCDD/Fs (28). Swedish otter populations have increased during the 1990s as the PCB levels in the environment have declined (29). Few studies have been made of wild mink or otter populations to link EDC exposure to reproductive outcome and little information is available about other possible environmental stressors that could have impacts. Therefore, the data are inadequate for linking the effects seen to an endocrine disruption mechanism.

Reproductive dysfunction in Baltic Sea seals
In the 1960s, populations of grey (*Halichoerus grypus*) and ringed (*Phoca hispida*) seals declined dramatically in the Baltic Sea (30,31). Female seals showed reproductive impairment including spontaneous abortion of seal pups, uterine occlusions and partial or complete sterility (32,33). The seals also showed adrenal cortex hyperplasia, skull lesions and malformed claws (31,34). The adrenal hyperplasia found in the seals is a clear indication of an endocrine-mediated effect. Seals from the Baltic were found to have high DDT and PCB concentrations and higher concentrations were found in diseased individuals (35). Methylsulfone-DDE (MeSO$_2$-DDE), a DDT metabolite, was also found in high concentrations in seals (36,37). Laboratory studies in mice have shown that MeSO$_2$-DDE is very toxic in the adrenal cortex (38) and is implicated in the adrenal effects seen in seals. Temporal studies of PCB and DDT concentrations in grey seals from the Swedish Baltic coast show declines after they were banned in the 1970s and 1980s, and seal populations have slowly increased in the same period.

In a semi-field study, captive harbor seals (*Phoca vitulina*) were fed either fish from the Wadden Sea (highly contaminated with PCB, DDT and other EDC) or fish from the Atlantic (low contamination). The seals fed Wadden sea fish showed a 50% reduction in reproductive success (39).

Mink have been used as a surrogate laboratory animal for seals when studying effects of EDCs, particularly PCB effects as both species have delayed implantation. Taken together, the evidence from laboratory studies in mink and various studies in seals is considered strong that the reproductive effects seen in Baltic seals are linked to their exposure to PCB. The link between adrenal hyperplasia and MeSO$_2$-DDE exposure is not sufficient to implicate this in the endocrine disruption that is seen. The endocrine disruption seen in seals seems to be associated with high PCB and DDT levels but mechanistic studies are needed to link this to reproductive impairment.
Conclusions

We can now return to the framework proposed by WHO (2) using the example of eggshell thinning. The hypothesis has two parts. The first is that eggshell thinning is caused by DDE exposure, which results in broken eggs. The second part is that this is due to an endocrine-mediated mechanism. How well do the data fill the five criteria in the framework to prove these two hypotheses? If we look at temporality, the period of high DDT use in North America and Europe coincides with the onset of severe population declines in many bird species and with eggshell thinning. The strength of association is strong between DDE exposure and the effects on eggshell thickness and similar effects were seen all over the globe. When considering consistency, the effects were seen in many species from many sites. The sensitivity to DDE-induced eggshell thinning varies among species and may be due to different mechanisms. Laboratory studies confirm the relationship between DDE exposure and eggshell thinning fulfilling the plausibility criteria. Recovery has been seen where many bird species sensitive to DDE-induced eggshell thinning have shown population increases and thicker eggshells with declining environmental DDT levels. The overall strength of evidence is therefore considered strong for the first part of the hypothesis. However, for the second part, linking the effect to an endocrine-mediated mechanism, the evidence is considered to be only moderate.

Table 1 shows the results of applying this framework to the five case studies presented here. As can be seen, all five give strong support for the first part of the hypothesis, that there are strong relationships between effects and exposure to a particular EDC. However, the second part of the hypothesis is not as well supported, in most cases due to the lack of mechanistic data.

In addressing the WHO framework, it is therefore difficult but possible to link effects seen in wildlife at the individual or population level to specific endocrine disrupters. This requires a combination of field, semi-field and laboratory studies. Coupling EDC exposure to an effect and that effect to an endocrine disruption mechanism is more difficult and has not been done conclusively for wild

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birds or mammals. To reach this goal requires mechanistic studies to determine how the EDC in question is acting on the endocrine systems of wildlife. This also requires a better foundation for understanding the physiology and endocrine systems of wildlife species.

References


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