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Review of the effects of endocrine-disrupting chemicals in birds*

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Abstract: There have been several case studies of the impact of chemical contaminants on birds at the level of individuals or populations. While many of the chemicals involved in these incidents have been classified as endocrine-disrupting chemicals or endocrine active substances (EASs) the mechanisms by which these chemicals affect birds are not clearly or fully understood.

INTRODUCTION

There are a number of reasons that some bird populations have declined significantly since prehistoric times, including habitat loss, hunting, climate change, and population genetics, among others. However, some of the effects have been caused by exposure to anthropogenic chemicals [1]. Birds, particularly piscivorous birds, have been exposed to a number of toxic, synthetic compounds, particularly persistent bioaccumulative organic compounds [2]. Specifically, the persistent chlorinated hydrocarbons have accumulated to the greatest concentrations and have been associated with the most severe adverse effects [3]. Organochlorine pesticides (such as dieldrin) and organophosphorus insecticides that are acutely toxic have caused widespread mortalities of adult birds. Other chemicals have had more subtle effects in reproduction, such as deformities and embryo lethality [2,4,5]. These effects, have, in turn, caused declines in populations [6,7]. These effects that have been referred to as endocrine-disrupting effects [8].

The most dramatic chemical effect on reproductive performance in birds was the result of eggshell thinning, caused by DDE, a degradation product of DDT [9–13]. Since limitations have been placed on the manufacture and use of these persistent widespread contaminants, concentrations in fish and birds have decreased [14,15]. Currently, concentrations of DDE in birds from many areas of North America have decreased below concentrations associated with eggshell thinning. As a result, populations of some fish-eating, water birds have increased rapidly [16]. However, other adverse effects, such as localized impairment of reproductive performance [17] and anatomical defects [1,18] persist. While exposure to certain organochlorine compounds, such as DDT and its degradation products, is associated with adverse effects, the mechanism of action is still not fully understood. It was not known if the currently observed effects were due to existing contaminants and would abate as concentrations decreased further or if the effects were caused by some, as yet unidentified chemicals. It has been speculated that

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the observed effects acted through mechanisms that modulated the endocrine system [19]. It was further speculated that some of the observed effects, such as the deformities would have been observed during the 1950s through the 1970s at equal or greater rates of incidence, but were masked by the effects of eggshell thinning. It had been suggested that since concentrations of all of the known toxic chemicals in the food, tissues, and eggs of birds were declining the observed effects might not be due to synthetic, organic compounds. The suite of effects observed did not seem to be the types that could all be explained by disease or nutrition or a genetic "founder effect" since populations of birds had been greatly reduced due to the effects of DDE. While this might explain some of the effects, such as birth defects, it was not likely that all of the effects could be explained by genetic deficiencies. It was also suggested that the effects might be due to new, nonpersistent pesticides that could have effects at small concentrations, but would leave little or no trace in eggs of afflicted adults.

The suite of effects, observed in wild populations of birds, especially in the North American Great Lakes and the Baltic Sea have been compared to those caused by exposure to certain halogenated hydrocarbons in laboratory exposures [1,2]. Many of the observed field effects could be reproduced in controlled laboratory studies at tissue concentrations in the same range as those observed in field-collected organisms [20]. Therefore, Giesy and coworkers [2] compared concentrations of 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) or TCDD equivalents (TCDD-EQ) in eggs of birds from the Great Lakes to concentrations known to cause effects. Based on concentrations observed in water birds and their eggs, it seemed unlikely that the PCDD and PCDF were responsible for the observed effects. These same effects were observed in birds exposed to polychlorinated biphenyls (PCBs) at concentrations similar to those observed [21]. However, a fairly poor correlation between the total concentrations of PCBs and egg lethality or birth defects was observed [17]. The weight of evidence demonstrated that there have been effects of these synthetic chemicals on birds. However, there is little evidence to support the conclusion that these effects are through modulation of the endocrine system. Furthermore, there is no evidence that the observed effects occur because any of these compounds act as estrogen agonist/antagonists.

The effects caused by synthetic chemicals on birds have been used to support what has been called the endocrine disruptor hypothesis [8]. Changes in reproduction, gender ratios, and eggshell thickness have been used to support a subhypothesis, the estrogen hypothesis. This hypothesis holds that the effects caused by exposure to persistent organic pollutants are caused by modulation of the endocrine system by these compounds acting as hormone agonists or mimics. In the case of estrogenic effects, it has been hypothesized that the adverse effects are caused by the xenoestrogen binding to the estrogen receptor (ER) and thus eliciting adverse effects.

To perform accurate ecological risk assessments it is always necessary to understand the mechanism of action. Only in this way can the effects of complex contaminant mixtures be interpreted and appropriate thresholds for protection be developed. It is unclear how classifying a compound as an "endocrine disruptor" assists in conducting an ecological risk assessment. What is required is an understanding of the mechanism of action, regardless of whether it involves direct or indirect action on the endocrine system or any endocrine-modulated effects at all. Here, we discuss some of the major effects observed in birds associated with exposure to environmental residues and discuss likely mechanisms of action. First, the processes of sexual differentiation and reproduction in birds are outlined with regard to their vulnerability to modulation by chemicals. Throughout this chapter, an attempt has been made to present and reconcile the results obtained from in vitro and in vivo laboratory studies, and field studies. The combined evidence from all these studies is crucial to understanding the complex phenomenon of endocrine modulation. The endocrine system of birds is described and the potential for xenoestrogens to affect these systems is examined. Some of the phenomena observed in birds that have been attributed to endocrine disruptors, especially chlorinated hydrocarbons or attributed to the effects of xenoestrogens are discussed. These include embryo-lethality, congenital deformities, skewed sex ratios, abnormal pairing behavior, and eggshell thinning.

EFFECTS OF SEX HORMONE MODULATORS ON SEXUAL DIFFERENTIATION

Gonadal differentiation in birds

In both birds and mammals, very early development is characterized by a sexually undifferentiated state [22]. Undifferentiated gonads are identical in "male" and "female" embryos/fetuses, and have the potential to develop into either state. The homogametic sex is the "default" sex, a differentiating hormone produced as a consequence of the "odd" chromosome causes the embryo/fetus to switch gender from the "default" sex. In mammals, the female is the homogametic sex (XX) "default" sex, the male is heterogametic (XY). The *Sry* region of the Y chromosome (the sex-determining region of the Y) encodes for maleness, its gene product is referred to as the H-Y antigen. The differentiating hormone for the mammalian testis is testosterone.

In birds, the homogametic default sex is male (ZZ), the heterogametic sex (ZW) is female. In birds the differentiating hormone for gonadal development is estrogen (c.f., testosterone in mammals). Female birds have an H-W antigen, analogous to the H-Y antigen in mammals, this gene product is the differentiation antigen for the heterogametic gonad (the avian ovary). Interestingly, this gene is not W-linked; it is present in both sexes and is activated by embryonic estrogen exposure [23]. It is, therefore, possible to cause avian sex-reversal from male to female, including expression of H-W antigen, by administration of estrogen to the genetically male egg during the critical period of embryonic development.

The early avian embryo has left and right Wolffian (preseminiferous tubules) and Müllerian ducts (preovarian ducts) which connect primordial gonads to the cloaca [24]. The Wolffian ducts serve as functional nephric ducts throughout embryonic development and normally regress and degenerate in females post-hatch. In males they are incorporated into the reproductive tract and are influenced by testicular hormones. Müllerian ducts regress in male embryos under the influence of testicular hormones. In females, the right Müllerian duct regresses such that only a small caudal remnant normally remains post-hatch, while the left duct develops into the primordial shell gland. In the absence of estrogen both gonads develop into testes, while during normal female development the left gonad develops into an ovary while the right gonad regresses [25].

Differentiation of brain and sexual behavior in birds

In mammal and bird behavior, neural substrates differentiate at the same time as, or after, the gonads [26], and are influenced by the hormonal secretions of the gonads. Adult behavior is influenced both by the organizational and activational effects of sex hormones. Organizational effects are permanent actions that establish the fundamental form (male or female) of an organ or behavior, they generally occur during critical periods of development. Activational effects are not permanent, are not limited to any critical period, and typically occur at or after puberty or sexual maturity. In both birds and mammals, estrogen is the primary differentiating hormone for behavior [27]. In male rats, the active differentiating hormone for copulatory behavior is estradiol, synthesized in the brain from testosterone supplied via the blood. In birds, ovarian estrogens directly feminize and demasculinize copulatory behaviors [28].

Early sex hormone administration can have profound and permanent sex-reversing effects on adult behavior in birds [29]. When male quail embryos (*Coturnix japonica*) are injected with 1 μg estradiol or 500 μg testosterone during a critical period of incubation, they can be completely behaviorally sex-reversed. As adults, they fail to mount, crow, or strut; they are completely demasculinized and are behaviorally indistinguishable from females [30]. This is due to a fundamental change in the neural substrate underlying behavior. The early organizational effect of estradiol confers a differential responsiveness to the activating effects of testosterone in adulthood: testosterone treatment restores copulation in castrated adult males, but is without effect in females. Female quail treated with an antiestrogen before hatching can be masculinized, and will mount other females as adults [29]. Therefore, during nor-

mal development of quail, an embryo develops as a behavioral male unless estrogen is present. Testosterone-induced organizational demasculinization requires the aromatization of testosterone to estradiol; the enzymatic aromatization activity in the embryonic quail brain is likely to be low, and thus protects male embryos from their endogenous secretions [31].

The sexual differentiation of behavior has also been extensively studied in the Zebra finch. This species exhibits sexual dimorphism of the brain and of behavior; normally only males sing, dance, and mount. The telencephalic nuclei (TN), higher vocal center (HVC), nucleus robustus archistriatalis (RA), nucleus magnocellularis of the anterior neostriatum (MAN), and area X of the lobus paraolfactorius (X) are larger and more extensively connected in males, which is essential for the learning and production of complex vocalizations [32]. The administration of exogenous estrogen to females during the first week post-hatch results in a profound organizational masculinization of brain and behavior [33–35]. This includes a neural masculinization of the TN, setting up a functional circuit in females similar to that in males enabling the learning and production of complex vocalizations [32]. When these females are stimulated with testosterone as adults, they engage in male behaviors, including singing and dancing [35]. Interestingly, males treated with estradiol during the first week post-hatch are demasculinized, and fail to mount as adults [35]. Thus, the pattern of behavioral sexual differentiation in the zebra finch is complex; estradiol treatment during the first week post-hatch masculinizes one behavior and demasculinizes another. A number of possible explanations for this have been proposed but for our purposes, it is sufficient to note that the process of sexual differentiation of behavior in this species is finely tuned, complex, and sensitive to estrogens.

The critical period for sexual differentiation of behavior varies with the biology of the bird species. The sex-reversing effects of early estrogen treatment on male Japanese quail behavior only occur if the treatment is before day 12 of the 18-day incubation [30], the estradiol-induced masculinization of the female zebra finch is only produced by treatment after hatching [35]. Quail are precocial (well-developed at hatching); zebra finches are altricial (poorly developed at hatching). The timing of sexual differentiation of behavior in these two species is consistent with the observation that precocial and altricial birds develop similarly, but hatch at different stages of development [29].

Susceptibility of birds to exogenous estrogens

There are several biological differences between birds and mammals that may confer differential sensitivity to estrogenic substances during sexual differentiation. In general, exposure of an avian embryo to exogenous estrogens during the critical period should have a greater adverse affect than would exposure of a mammalian fetus to estrogens during the same critical period. This is because in birds, estrogen is the differentiating hormone for both gonads and behavior. In contrast, estrogen is not the differentiating hormone for gonadal development in mammals. While exposure of the mammalian fetus to estrogen may cause changes in the development of sexual characteristics, the changes are not as profound as those that would be caused by testosterone exposure.

For the avian embryo to be exposed to exogenous estrogens during the critical period, these compounds must be passed from the female bird to her eggs. Some estrogenic pollutants, such as DDT, are readily transferred to lipid-rich yolk. It is possible for fish-eating birds to acquire substantial body burdens of such contaminants from their diet, and to transfer these to the egg yolk. However, if estrogenic substances do not pass from female to egg, the avian embryo is more protected from adverse effects than the mammalian fetus because avian embryos are isolated from the maternal blood stream during gonadal sexual differentiation.

Avian embryos may be more sensitive than mammalian fetuses to estrogenic chemicals, which require metabolic activation [25]. Water-soluble metabolic products of xenobiotics are not excreted from the avian egg, but remain in the blood circulation throughout incubation. The phase 1 metabolites of many organochlorine contaminants are considerably more estrogenic than the parent compounds [36,37]. It is possible that during the last half of incubation, the avian embryo may be capable of mixed-

function oxygenase metabolism, including hydroxylation of organochlorines, and yet still be within the critical period for sexual differentiation of brain and behavior. This would be an interesting topic for future research.

Modulation of endocrine function during reproduction or development in birds by environmental chemicals: Laboratory evidence

Several persistent organochlorine pollutants exhibit estrogenic activity, and can influence reproduction and avian embryonic development. Kepone has a binding affinity for chicken estrogen receptor that is about 5000-fold less than that of 17β-estradiol [38]. Kepone stimulates growth and dramatic morphological changes in the oviduct of immature Japanese quail when administered at 160 μg/g in the diet [38]. Chronic ingestion of kepone at relatively great concentrations produces reproductive impairment, decreased eggshell quality, and decreased chick survival in several avian species [39].

o,p′-DDT and the di-demethylated metabolite of methoxychlor also bind to the estrogen receptor and produce estrogenic effects in vivo [40]. Altered gonadal development was observed following injection of *o,p′*-DDT or methoxychlor into gull eggs on day 1 of incubation [41,42]. The male gonads were feminized, right oviducts persisted in surviving females, the gross morphology of *o,p′*-DDT or methoxychlor injected eggs was intermediate between controls and estradiol injected eggs. It is difficult to judge the functional significance of these results since the criteria for feminization was a small histological change (i.e., a localization of primordial germ cells in a thickened cortex on the surface of the left testis). Ideally, the egg injection study with *o,p′*-DDT should be repeated, and hatchlings should be grown to maturity and their reproductive health assessed.

Organochlorine chemicals have also altered avian reproductive behavior in controlled studies. Adult ring doves fed a mixture of organochlorines exhibited alterations in hormone concentrations and reproductive behavior [43]. Consumption of contaminants led to a reduction and/or delay in behaviorally induced increases of sex hormones, contaminated females failed to respond to male courtship behaviors, and pairs receiving the highest dosage spent less time feeding the young. There was a marked dose-related decrease in fledging success, and the breeding cycle was greatly asynchronous in treated birds. The administration of a polychlorinated biphenyl (PCB) mixture to adult breeder doves resulted in aberrant incubation [44] and courtship [45] behaviors. PCB-dosed females were particularly affected in the later experiment, performing only a small number of courtship behaviors, resulting in a severe impairment of reproductive success.

Other environmental contaminants have also had a negative impact on avian reproduction due to endocrine disruption. Oral administration of crude oil caused elevated plasma corticosterone and thyroxine levels in herring gull and black guillemot nestlings, apparently related to depressed growth [46]. Exposure to the organophosphorus insecticide parathion may also impact avian incubation behavior and reproductive success [47,48], although such impacts are likely to be of a more acute and transient nature than organochlorine-induced reproductive impairment. Natural environmental estrogens, such as phytoestrogens in the diet, can also influence avian reproduction. California quail experienced a delayed onset of reproduction and decreased egg production when a plant extract containing isoflavones such as genistein was incorporated into their diet [49].

Sex ratio skew and female/female pairings in gull populations

An increase in female/female pairings in gull populations in DDT-contaminated regions is the most commonly cited evidence of organochlorine-induced estrogenic effects occurring in birds [42,50]. Due to difficulties encountered in accurately observing and sexing all birds in gull colonies, the incidence of female/female pairings is estimated by the number of nests containing abnormally large numbers of eggs ("supernormal clutches"). A single female gull typically lays 1 to 3 eggs. Situations by which 4-egg clutches arise are variable, unclear, and often not associated with female/female pairings [51].

Nests with five or more eggs are almost always the result of multiple-female associations. The number of nests with five or more eggs provides a reasonable assessment of the incidence of female/female pairing in a colony, although it probably under estimates the actual incidence.

Generally, female/female associations in gull colonies arise due to shortage of breeding males, as an increased incidence of supernormal clutches can be induced by removal of males [52]. While some supernormal clutches arise from female/female pairs, others are due to polygynous trios of two females and one male [53]. Females may associate with one another when males are unavailable to increase their probability of reproductive success, however slightly. A female may be able to achieve egg fertilization via copulation with a promiscuous male, but without a partner, will be unable to raise a chick as two nest attendants are necessary in gull colonies. Females may also associate to avoid aggression directed toward unmated birds in order to stay with the colony, and perhaps to increase their likelihood of obtaining a mate in future years [54]. Some studies demonstrate low reproductive success of supernormal clutches [55,56], others have demonstrated fledged offspring from supernormal clutches [57].

In gull colonies, a sex ratio skewed toward females can occur in several ways. It is quite common in rapidly expanding areas [58], as female gulls are less philopatric than males and are more common emigrants [59]. A skewed sex ratio can also occur if there is differential male mortality in a population, or in situations where there is a differential failure of male recruitment to the breeding population. Sex ratios skewed towards females were documented in several gull colonies during the 1960s and 1970s. The most dramatic and well-documented example occurred in the adult western gull population on Santa Barbara Island between 1972 and 1978 [56], the sex ratio was estimated to be 0.67 males/female. A supernormal clutch incidence of 0.6 to 1 % was observed in herring gulls inhabiting northeastern Lake Michigan during the period from1978 to 1981 [54,60]. Both the California and Great Lakes populations of gulls were exposed to relatively great concentrations of organochlorine compounds, including DDT, from the 1950s until the 1970s [41]. Several historical studies investigated the occurrence of supernormal clutches in the Laridae, utilizing literature sources and museum specimens, in order to determine whether incidences have actually changed in the pre- and post-DDT era. The incidence of supernormal clutches has actually decreased significantly for many species of terns throughout the United States [61]. Supernormal clutch incidence only increased significantly in three Laridae species since 1950: western gulls, Great Lakes herring gulls, and Caspian terns in the United States. Supernormal clutches were a regular occurrence in ring-billed and California gulls prior to the DDT era, and their occurrence has not changed over time [62]. Supernormal clutches were not found regularly in western or herring gulls until after 1950, since that time the sex ratio for their populations as a whole has changed dramatically towards an excess of females. Therefore, it appears that the shortage of males at breeding colonies resulted from a low male/female ratio in the population and not from a failure of feminized males to breed, since few of the museum specimens from which the sex ratios were calculated were collected from breeding colonies.

A decrease in the sex ratio of western and herring gulls could be due to a differential mortality, it is possible that male gulls are more susceptible to poisoning from persistent organochlorines. Male western gulls weigh about 25 % more than females, and feed higher up on the food chain [63]. Also, male gulls do not have the ability to excrete lipophilic contaminants by egg-laying. Therefore, males may accumulate greater body burdens of toxicants during their lifetimes than females. Alternatively, the cause of differential male mortality may not involve contaminants at all.

It has been suggested that the skewed sex ratios observed in western and herring gulls might have been caused by estrogenic environmental contaminants, either by differential male mortality or by feminization of male embryos resulting in chemical sterilization and failed recruitment into the breeding population [42]. While this is plausible, there is no direct evidence to support it. The hypothesis that females might have paired with the wrong sex due to chemical-induced masculinization is not supported by a detailed behavioral study that found no differences in behavior between females paired with females and those paired with males [64]. An attempt to correlate gonadal feminization with organochlorine contamination in Glaucous-winged gulls in Puget Sound was also inconclusive [42]. Thirty-one

adult females from colonies spanning a range of contamination were trapped and sacrificed for gonadal inspection. Interestingly, the length of the right oviducts was correlated with the predicted degree of chemical contamination. However, the significance of the data is rather unclear, as all birds were successfully incubating clutches. Further, the most severe category was rated as greater than 10 mm length, while the literature indicates that a vestigial right oviduct of 9 to 10 mm is normal in the herring gull [65]. Unfortunately, although eggs were collected from the nests, no chemical data were presented. Finally, 4-egg clutches were considered "supernormal", but clutches of this size usually do not result from female-female associations [52] and are of dubious significance to the argument.

In conclusion, there is good evidence that there has been a fundamental change in the sex ratio of several North American gull populations in the post-DDT era, resulting in an overabundance of females in some colonies. The observations that the colonies most affected were in areas of great DDT contamination, and that a few DDT congeners have produced abnormal gonadal development in laboratory studies, support the hypothesis that environmental contaminants may have played a role in the sex ratio skew. However, direct evidence for that hypothesis is lacking, and any mechanism which could cause differential male mortality in gull populations could be a viable alternative explanation worthy of investigation.

MECHANISM OF DDE-INDUCED EGGSHELL THINNING

During the 1960s and 1970s, when DDT was in the North American environment at greater concentrations, populations of several sensitive bird species declined when individuals were unable to successfully incubate eggs because of abnormally thin shells [66]. Many of these species, such as the double-crested cormorant, experienced dramatic population increases after DDT was banned in the United States and environmental concentrations declined [67,68]. The eggshell-thinning effect of *o,p′*-DDT and its potent, stable metabolite *p,p′*-DDE in sensitive species is well known, even to the lay public. In fact, the contribution of DDT to population declines in bird species such as brown pelicans, peregrine falcons, and bald eagles is probably the most famous incident in wildlife ecotoxicology. It is, therefore, quite ironic that the mechanism by which DDTs cause eggshell thinning is still not understood.

Several possible mechanisms of DDE-induced eggshell thinning have been suggested however the mechanism appears to vary among species [66,69]. It is also difficult to study the mechanism as the most convenient laboratory species, including chicken and quail, are insensitive to this effect [70]. Research in this area within the last decade has been performed almost exclusively by one individual using one sensitive avian species [71–83].

An examination of the structure of DDE-induced thin eggshells can provide valuable clues about possible mechanisms of malformation. Some postulated mechanisms such as premature termination of shell formation, premature oviposition, effects on the protein matrix of the shell, effects on initiation sites of shell formation, or enhancement of shell growth inhibitors could be investigated by examining shell structure and composition. Indeed, abnormalities are observed in DDE-affected shells of several species. Species which produce eggs with a chalky valerite cover, including pelicans, cormorants, shags and gannets, produce eggs with a much reduced or absent cover following DDE exposure [69,84]. In these species, the shell-forming process is most impacted by DDE towards its termination. Other birds such as the great black-backed gull [69] and the grey heron [85] show a general reduction in all shell layers following DDE exposure. Changes in mineral composition of eggshells following DDE treatment have seldom been investigated [86].

Attempts to determine the mechanism of DDE-induced eggshell thinning have been based upon the a priori assumption that an alteration in Ca^{2+} metabolism of the shell gland is involved [81]. In two species of ducks, the effect of DDE administered in vivo has been localized to an interference of the translocation of Ca^{2+} from the shell gland mucosa cells to the shell gland cavity [75]. Calcium binding and transport have been studied, and the involvement of progesterone, prostaglandins, and calmodulin has been considered. There is substantial in vivo evidence that the shell gland actively transports Ca^{2+}

from the plasma into its lumen [87], and it has been suggested that this translocation of Ca^{2+} is driven by an ATP-dependent calcium "pump" which is inhibited by DDE [72]. Early reports indicated that calcium-dependent adenosine triphosphatase (Ca-ATPase) was reduced in the shell gland mucosa of DDE-treated birds which exhibited eggshell thinning [88,89]. Reduction of Ca-ATPase activity occurred only in DDE-sensitive species, and was considered a more likely candidate for the causation of eggshell thinning since such transport enzymes do not appear to be present in excess and a partial inhibition is generally accompanied by a parallel reduction in ion transport [88]. In ducks, the total content of calcium in the secreted lumen fluid was decreased following DDE treatment while the calcium content of the mucosal gland cells was increased, indicating that calcium translocation between the blood and shell gland mucosa was not impaired, but that translocation from the mucosa to the lumen was affected [75]. Detailed studies of the subcellular location and the timing of DDE-related effects on ATP-dependent Ca^{2+} uptake by and Ca^{2+}-Mg^{2+}-activated ATPase activity in the shell gland revealed complex relationships. DDE had no effect on the Ca^{2+} binding to mucosal homogenate of nonsecreting ducks (i.e., ducks with a fully calcified egg in the uterus), while DDE affected Ca^{2+} binding in actively "secreting" (i.e., in the process of forming a shell) ducks by decreasing mucosal microsomal binding, but increasing mucosal mitochondrial binding [75]. It was suggested that this effect of DDE might involve a decrease in the uptake of Ca^{2+} in secreting granules or in an interference with the stimulus-secretion coupling mechanism of calcium. Further studies revealed that DDE had an inhibitory effect on Ca^{2+} uptake of secreting shell gland mucosa, but there was generally no accompanying reduction of the Ca^{2+}-Mg^{2+}-ATPase activity [76]. While DDE markedly inhibited Ca^{2+}-Mg^{2+}-ATPase activity when added in vitro, when DDE was administered in vivo it often, but not always, resulted in an absolute or functional increase in Ca^{2+}-Mg^{2+}-ATPase activity despite a reduction in the rate of Ca^{2+} uptake. This indicates that DDE had an "uncoupling" effect on Ca^{2+} uptake and Ca^{2+}-Mg^{2+}-ATPase activity.

The current hypothesis regarding the mechanism of DDE-induced eggshell thinning involves an inhibition of prostaglandins (PGs) by the shell gland mucosa. Progesterone stimulated the synthesis of PGs in the eggshell gland mucosa of estrogen-primed chickens, and PGs play an important role in the control and regulation of reproduction in birds [82]. PG synthesis is decreased by *p,p′*-DDE in duck shell gland mucosa, both in in vitro experiments and following in vivo exposure [82]. PG synthesis was not inhibited by *p,p′*-DDT or *o,p′*-DDE in in vitro experiments, which indicates specificity between the proposed cause (PG synthesis inhibition) and the most potent eggshell thinning chemical (*p,p′*-DDE). However, in in vitro assays, a PCB Aroclor mixture (1242) was also an extremely potent PG synthesis inhibitor in shell gland mucosa. PCBs are not, however, potent eggshell thinning chemicals. It is unfortunate that the researchers did not conduct a parallel feeding trial with PCB 1242, in addition to their feeding trial with *p,p′*-DDE, in order to compare in vivo effects on PG synthesis. Also, the mechanism by which inhibition of PG synthesis might cause thin eggshells has not been definitively established. Some researchers have hypothesized that a furosemide-insensitive, PG-stimulated HCO_3^- transport could be inhibited in the shell gland mucosa of DDE-treated ducks, but further experiments have not supported that hypothesis [79].

Research into the mechanism of DDE-induced eggshell thinning has shown that the phenomenon is quite complex. Eggshell thinning is associated with a decreased quantity of calcium in affected eggs, and in the mallard duck the effect of DDE has been associated with a decreased transport of calcium from the eggshell gland mucosa to the lumen fluid. Treatment of birds with DDE has been associated with a variety of biochemical changes that could be related to changes in calcium transport. Many of these biochemical endpoints are interrelated, and it is difficult to determine which are the direct targets of DDE and which are merely coinfluenced by its action. The situation is further complicated by the fact that sensitivities to DDE-induced eggshell thinning vary among avian species, and in fact different mechanisms might be causing eggshell thinning in different species as evidenced by different gross eggshell defects.

Recent studies in New Zealand have highlighted the fact that eggshell thinning can be attributed to a variety of environmental factors. Investigations were begun into possible causes of eggshell thinning in Northern royal albatross nesting on one of New Zealand's sub-Antarctic islands. While the eggshell thinning observed was of the right type and magnitude to suggest a xenobiotic etiology the impact was limited to a single colony within the species range. Chemical analysis revealed background concentrations of persistent organochlorines including DDTs, but the concentrations measured were similar to those measured in other successful colonies [90]. Further investigation indicated that the cause of the shell thinning was crowding stress bought on by catastrophic habitat degradation, vegetation removal, and subsequent crowding stress [91]. This study illustrates both the complex nature of the eggshell thinning phenomenon and the degree of caution needed in investigating the relationship between xenobiotics and bird reproduction.

DEFORMITIES

Of the adverse effects observed to occur in colonial water birds of the North American Great Lakes region, the most obvious and that which can be most directly related to survival of individuals and populations are embryo lethality and developmental deformities. In the region, most of the embryos or chicks, which die during early development, have developmental deformities [92,93], particularly abnormalities which are of ectodermal origin [94]. One of the best-documented abnormalities, correlated with concentrations of planar chlorinated hydrocarbons (PCHs) in bird eggs, is the crossed-bill syndrome [93]. This suite of conditions found in Great Lakes wildlife has been named the Great Lakes embryo mortality, edema and deformity syndrome (GLEMEDS) [93]. This syndrome mimics chick edema disease, observed in the offspring of hens exposed to polychlorinated-dibenzodioxins and -dibenzofurans [95].

The few available case studies illustrating the effects of these chemicals are the reports of the Canadian Wildlife Service on studies performed on herring gulls living on Lake Ontario during the 1970s. In the late 1960s, anecdotal evidence circulated among field biologists of poor rates of hatching of Lake Ontario herring gull eggs [96]. Official Canadian Wildlife Service surveys began in 1971. Hatching rates of less than 20 % were found at some colonies in Lake Ontario. Initial examination of herring gull eggs and eggs of other species in Lake Ontario documented the presence of DDT and PCBs. However, analytical techniques at the time were incapable of measuring polychlorinated-dioxins (PCDDs), -furans (PCDFs), and the structurally similar PCB congeners. The characteristic symptoms in surviving chicks were similar to those of chick edema disease. Subsequent reanalysis in the late 1980s of a variety of eggs that had been collected from herring gull colonies in Lake Ontario in the 1970s were found to contain 1–3 μg/kg, wet wt. of TCDD. This chemically caused epizootic in Lake Ontario is probably the best-documented example of *dioxin-caused* effects on wildlife. During the last decade, the symptoms of chick edema disease and GLEMEDS have decreased significantly in the herring gulls of Lake Ontario [93], but more subtle, biochemical effects persist in all species of fish-eating birds inhabiting the Great Lakes [50].

Studies of nesting Forsters terns, conducted in Green Bay from 1983 to 1988 [4,97], and on double-crested cormorants and Caspian terns [18,92] demonstrated a similar suite of biological effects, but implicate PCHs other than TCDD. In Green Bay, Kubiak and coworkers found a variety of developmental deformities in the embryos and chicks of Forster's terns including growth deficiencies, deformities, and behavioral differences in parental care of eggs compared to an inland control colony where exposures were significantly less than on Green Bay [4]. Extrinsic adult behavioral abnormalities of inconsistent incubation led to a four-day-longer incubation period than the reference colony. Reciprocal transplant studies of eggs resulted in similar time delays ascribed to toxic substances in the eggs. That study suggested widespread, complex contaminant effects on the reproductive cycle, such as longer incubation times, smaller individuals, and wasting syndrome in those that did hatch. Similar, but less acute deformities, have been observed in double-crested cormorants and Caspian terns in upper Green

Bay where the total concentration of 2,3,7,8-TCDD equivalents (TCDD-EQ) ranged from 175 ng/kg wet wt. to greater than 440 ng/kg [18,92]. The majority (>90 %) of the TCDD-EQ in the eggs of cormorants and terns in the Great Lakes was due to non-*ortho*-substituted PCBs [98,99], rather than PCDD or PCDF, which accounted for between 2 and 9 % (12–22 ng/kg, wet wt.) of TCDD-EQ measured in water bird eggs in Lakes Superior, Huron, and Michigan. The primary contributions to the TCDD-EQ were due to the *dioxin-like* non-*ortho*-substituted PCBs, especially non-*ortho*-chlorinated congeners 126, 77, 169 and mono-*ortho*-chlorinated congeners 105 and 118. The understanding that dioxin-like effects in fish-eating, colonial water birds are due largely to non-*ortho*-substituted PCBs is an emerging consensus worldwide, except near TCDD point sources [100,101].

Available evidence supports the hypothesis that most, if not all, TCDD effects are mediated through the AhR [102,103], a cytosolic receptor protein that was first discovered by Poland and coworkers [104]. The AhR signaling transcription pathway is initiated by TCDD diffusion into the cell, where it binds with high affinity to the cytosolic AhR protein complex, which also includes heat shock protein 90 (Hsp90) and a 38-kDa, immunophilin-related protein [105,106]. The ligand binding activates AhR and stimulates the dissociation of AhR-associated proteins. The ligand-receptor complex is subsequently translocated into the nucleus, where it dimerizes with AhR nuclear translocator (ARNT) [103,107]. The heterodimers are capable of recognizing and binding DNA at the consensus sequence, GCGTG, of dioxin responsive elements (DREs) [108,109]. This action either increases or decreases the transcription of target genes [110,111], including cytochrome P450 (CYP1A1, CYP1A2) [112,113], NAD(P)H:quinone reductase [114], class 3 aldehyde dehydrogenase and glutathione S-transferase. The ARNT protein, however, does not function uniquely in the AhR signaling pathway, but also pairs with HIF-1α (hypoxia inducible factor 1α) to regulate genes active in response to low oxygen stress [115–117]. HIF-1α is continuously synthesized and degraded under normal oxygen tension. Hypoxic conditions inhibit the degradation of HIF-1α by the ubiquitin proteasome system, and triggers the nuclear localization of HIF-1α [118–120].

AhR, ARNT, and HIF-1α belong to bHLH (basic-helix-loop-helix)/PAS protein family. The bHLH motif is characteristic of a family of proteins that function as modulators of cell proliferation and differentiation. PAS proteins are found in representative organisms of all five kingdoms and may play a role in determining target gene specificity [121]. PAS proteins are involved in development and differentiation (Sim group, trachealess) [122,123], regulation of circadian clocks (Per, CLOCK) [124–125], sensing and responding to oxygen tension (HIF-1α, EPAS-1/HLF) [117,126], and steroid receptor signaling (SRC-1) [127]. The myogenic bHLH proteins autoregulate their own expression and cross-regulate the expression of other family members [128], since the secondary dimerization surface of the PAS domain can provide the specificity for dimerization among PAS/bHLH proteins [129]. PAS proteins, therefore, may behave in a way similar to the myogenic bHLH proteins and interact with each other through the PAS domain. Since ARNT has been found to dimerize with many PAS proteins such as AhR, Sim1, Sim2, HIF-1α, and EPAS-1 [126,130–133], it may act as a central regulator of PAS protein-dependent pathways.

Since many of the effects that have been observed in wildlife and used as support of the endocrine disruptor hypothesis are thought to be caused by TCDD and structurally similar, synthetic halogenated hydrocarbons, it is important to understand the mechanism of action, as it relates to the endocrine disruptor hypothesis. While the effects caused by TCDD can be classified as an effect on signal transduction, these effects are not covered by the narrow definition of receptor-mediated effects of steroid hormones. Thus, these effects would not be identified in a screening process so narrowly defined as to include direct receptor-mediated effects on the estrogen, androgen, or thyroid receptors.

Thyroid hormone and vitamin A

In addition to the primary steroid hormones, thyroxine and vitamin A are crucial to normal development of sexual and immune functions. Altered concentrations of thyroxin and vitamin A are frequently

reported to co-occur with embryonic abnormalities in populations exposed to some synthetic halogenated hydrocarbons [93]. Individuals from these exposed populations have been observed to have altered sexual development [42], sexual dysfunction as adults [19], and immune system suppression [134,135]. The observations on adult sexual dysfunction are especially significant since young, which appear to be normal while raised by intoxicated parents, may become reproductively dysfunctional when mature [136,137]. Poor reproductive efficiencies and opportunistic diseases are characteristic of wild animals in exposed populations of the Great Lakes.

Vitamin A (retinol) has many functions in animals, such as in embryonic development, vision, maintenance of dermally derived tissues, immune competence, hematopoiesis, and reproduction. Vitamin A is necessary for normal embryonic development [138,139] and, thus, changes in the status of vitamin A in the plasma or liver may be responsible for the birth defects observed in birds, which have been exposed to PCHs. An inverse correlation has been observed between the concentration of vitamin A in serum and concentrations of PCHs in tissues of birds from Great Lakes. Both vitamin A and its storage form in the liver (retinal palmitate) were depleted in birds exposed to sublethal doses of the dioxin-like PCB congener 77 [140]. There are effects of PCH on concentrations of vitamin A in both the blood and liver. These effects are thought to be due to at least two processes. In blood, hydroxylated PCB metabolites bind to the carrier protein transthyretin [134,141,142]. Hydroxylated PCBs have been shown to be retained in the blood of birds [143]. In the liver, induction of hepatic enzymes involved in the degradative and mobilization pathways of vitamin A, such as, such as acyl-CoA:retinol acyltransferase and uridine diphosphate glucuronosyl transferase (UDPGT) are thought to alter the metabolic pathways involved in the storage and mobilization of vitamin A, which results in the observed depletion of retinols in the liver.

2,3,7,8-TCDD is known to have effects on both male and female steroid hormones. For instance, 2,3,7,8-TCDD has both estrogenic and antiestrogenic effects, in different tissues, depending on timing of exposures during development. Furthermore, 2,3,7,8-TCDD is a potent thyroxine agonist, which may account for its ability to cause wasting syndrome in homeotherms. The induction of the mixed function monooxygenase system can also reduce the concentrations of circulating steroid hormones, which can have adverse effects on the reproduction of wildlife [144].

Thyroid hormone, is an important regulator of development and metabolism and can be influenced by PCHs [134,139]. There are several possible mechanisms for the observed effects on circulating triiodo thyroxin (T_3) and thyroxin (T_4). First, hydroxy-substituted PCB congeners have been observed to displace T_4 from its carrier protein, transthyretin (TTR; prealbumin), which results in effects similar to thyroxin deficiency [141,142,145]. PCHs can induce UDPGT activity in the liver, which then decreases the concentration of TTR in the blood. Concentrations of TTR are not determined directly in the plasma, but rather, T_4 binding capacity is measured. Therefore, it is not possible to distinguish which of the two mechanisms may be causing the observed effects. TCDD also mimics the effects of thyroxine as a key metamorphosis signal during maturation [146]. 2,3,7,8-TCDD has also been shown to down-regulate the epidermal growth factor (EGF) receptor [147], which may result in disruption of the patterns of embryonic development at critical stages.

ALTERATIONS IN AVIAN THYROID HISTOLOGY BY GOITEROGENS

Goiter, an enlargement of the thyroid gland without hyperthyroidism, is endemic in Great Lakes wildlife [148]. Goiter is commonly caused by an iodine deficiency, although chemicals or defects that interfere with thyroid hormone synthesis or regulation can also cause the disease. Goiter can have a histological appearance which varies depending upon its cause. Avian goiter resulting from iodine deficiency is characterized by epithelial cell hyperplasia [149]. Epithelial cells are cuboidal to columnar, and the colloid is reduced and pale-staining. PCBs can cause goiter in animals by competitively binding to carrier proteins, such that thyroid hormones are not bound and are cleared more rapidly from the body. When there is a relative lack of thyroid hormones in the body, thyroid stimulating hormone (TSH)

can stimulate thyroid hypertrophy or hyperplasia. While mammalian goiter caused by PCBs is a hyperplastic goiter which resembles that caused by iodine deficiency, the histological picture in birds is quite different. In birds, PCB treatment results in hypothyroidism, and a large-colloid goiter [140]. Thyroid weight, follicle size, and colloid are all increased, and epithelial cells are flattened. At great PCB doses, thyroid atrophy is observed [150]. The types of goiter induced by other organochlorine contaminants are more variable. DDT and DDE have produced a hypothyroidism in Japanese quail [151], but an epithelial cell hyperplasia in the pigeon [152]. Dieldrin produced an epithelial cell hyperplasia in the pigeon as well [152].

Herring gulls in the Great Lakes had enlarged thyroids when compared to a control colony in the Bay of Fundy [148]. The observed goiter was characterized by epithelial cell hyperplasia, few columnar epithelial cells, a diffuse microfollicular structure, and scant or absent luminal colloid. Since various etiologic agents produce different histological appearances in birds, histopathology can be useful in determining the cause of goiter in birds. It is clear that, in the case of the Great Lakes herring gulls, PCBs were not the cause of the observed goiter. While it is possible that DDE or dieldrin could have been a contributing factor, it is also possible that iodine deficiency or goiterogens in the forage could have been involved.

CONCLUSIONS

The stage in early life during which the sexual differentiation of gonads, brain, and behavior occurs is probably the most sensitive period to the influence of endocrine modulation by xenobiotics in birds. Exposure to relatively small quantities of exogenous hormones during the critical periods for sexual differentiation can result in severe and permanent alterations in gonadal and/or neural structure and in subsequent adult reproductive behavior. Alterations in gonadal structure, reproductive behaviors, and reproductive success have been experimentally induced by administration of exogenous hormones during critical developmental periods. In general, the administration of relatively great concentrations of organochlorine chemicals is required to elicit similar albeit less dramatic effects. While it is plausible that female/female pairings and low male/female ratios in gull populations could be related to environmental contaminants and endocrine modulation, any mechanism that could cause differential male mortality in gull populations may be a viable alternate explanation for these phenomena.

The mechanism by which DDE induces eggshell thinning in sensitive avian species continues to be investigated. It is quite possible that the mechanism may be different among various avian species. The quantity of calcium is decreased in thin eggshells, and a decreased transport of calcium from the eggshell gland mucosa to the lumen fluid has been implicated as the cause of eggshell thinning in at least one sensitive avian species. The modulation of calcium homeostasis is a mechanism by which xenobiotics could impact eggshell development. The possible interference of DDE with calcium transport enzymes or calcium regulators such as calmodulin, progesterone, or prostaglandins has been investigated, and the complex interplay of these regulatory factors have made it difficult to isolate the primary target of eggshell-thinning xenobiotics such as DDE.

Goiter is an endemic disease in some avian wildlife populations. It has been postulated that goiter may in some cases be caused by the modulation of thyroid hormone homeostasis by xenobiotic chemicals in the environment. Goiter has been experimentally induced in birds by the administration of organochlorine contaminants. The histological presentation of goiter can vary substantially depending upon the etiologic agent responsible. Histological evidence can be used to infer that PCBs were not the cause of goiter observed in Great Lakes herring gulls [148]. The observed goiter was characterized by epithelial cell hyperplasia with scant or absent luminal colloid, in marked contrast to the large colloid goiter commonly observed in birds experimentally treated with PCBs.

Regardless of the mechanism, it is clear that some synthetic organochlorine compounds have had an adverse effect on some species of birds in some locations. Some of these effects were due to the fact that these compounds are persistent and able to bioaccumulate and biomagnify into organisms at the top

of the food chain. In some cases these were sensitive birds. There has been much discussion of the "endocrine disruptor hypothesis". As far as ecological risk assessments are concerned, this is somewhat of an artificial classification. While it is always important to know the mechanism of action of a compound, it is inappropriate to determine if a compound is or is not hazardous based on a single or a few mechanisms of action. The discussion of mechanisms of action presented here should make it evident that the simple classification of a compound as an endocrine disruptor or not is not very helpful in conducting the risk assessment. Specifically, classifying compounds on their ability to bind to specific steroid hormone receptors would not be very useful in screening compounds or providing the mechanistic information necessary to develop appropriate biomarkers or to interpret the potential effects of complex mixtures of compounds. There are many possible mechanisms through which persistent and bioaccumulative compounds can adversely affect organisms.

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