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A REVIEW OF CHEMICALLY-INDUCED ALTERATIONS IN THYROID AND VITAMIN A STATUS FROM FIELD STUDIES OF WILDLIFE AND FISH

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ABSTRACT: This paper reviews 22 published field studies that have found an association between exposure to environmental contaminants and alterations in thyroid gland structure, circulating thyroid hormones and vitamin A (retinoid) status in free-ranging populations of wildlife and fish. Vitamin A and thyroid hormones play critical roles during development, growth and function throughout life. Studies of captive wildlife and laboratory studies support a relationship between alterations in thyroid hormones and vitamin A status and exposure to dioxins, furans, and planar polychlorinated biphenyls, which bind to the aryl hydrocarbon receptor. Some studies have found adverse health effects in wildlife associated with exposure to polyhalogenated aromatic hydrocarbons and altered thyroid and retinoid status including: decreased reproductive success, immune system changes, dermatologic abnormalities and developmental deformities. A direct causal relationship between these effects and thyroid and retinoid changes has not been demonstrated. Field researchers studying the responses to these synthetic chemicals in wildlife and fish should include measurement of thyroid hormones and retinoids and histological examination of the thyroid gland in their study design as biomarkers of exposure to these chemicals in the environment.

Key words: Dioxin, fish, organochlorines, polychlorinated biphenyls, retinoids, thyroid, vitamin A, wildlife.

INTRODUCTION

It is now widely accepted that free-ranging populations of wildlife and fish have been impacted by exposure to man-made chemicals in the environment that can interfere with the development and function of the reproductive, endocrine, immune and nervous systems (Colborn et al., 1993; Rolland et al., 1995, 1997). These chemicals are referred to as "endocrine disruptors" and act by interfering with hormonal and other cell messaging systems (Colborn and Clement, 1992). Exposure to these chemicals during development and differentiation of major organ systems may result in irreversible physiological, morphological, and behavioral changes by altering the hormones that control the course of development. Referred to as organizational changes (Guillette et al., 1995), these alterations are of particular concern because developing organisms are more likely to exhibit effects at chronic environmentally-relevant exposure levels than adult organisms, which may not be impacted at all (Bern, 1992).

Important clues to the actions of endocrine disrupting chemicals have come from field studies of wildlife and fish. Many of these observations involve disruption that has occurred during embryonic development and sensitive early life stages resulting in impaired reproduction and developmental abnormalities in the offspring of exposed parents (Guillette, 1995). Some of the anomalies reported to date include: developmental deformities and wasting in fish-eating birds in the Great Lakes (Hoffman et al., 1987; Fox et al., 1991; Gilbertson et al., 1991; Giesy et al., 1994); reproductive impairment in bald eagles (*Haliaeetus leucocephalus*) (Bowerman et al., 1995) and wood ducks (*Aix sponsa*) (White and Hoffman, 1995); abnormalities of sexual development and reproductive hormone levels in alligators (Guillette et al., 1994); histological abnormalities of the thyroid gland in Great Lakes herring gulls (*Larus argentatus*) (Moccia et al., 1986)

and salmonids (Moccia et al., 1981); altered reproductive hormone levels and changes in secondary sex characteristics in fish (Munkittrick et al., 1991); altered immune system characteristics in marine mammals (De Swart et al., 1994; Lahvis et al., 1995); and altered neurological development in great blue herons (*Ardea herodias*) (Henschel et al., 1995).

Although well over 50 synthetic chemicals have been identified as endocrine disruptors, much of the field ecotoxicological research has focused on the persistent, lipophilic polyhalogenated aromatic hydrocarbons (PHAHs), which are ubiquitous environmental contaminants. Some of the most studied of these compounds are the toxic congeners of the polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and the planar polychlorinated biphenyls (PCBs) that share the ability to bind to the Ah receptor (aryl hydrocarbon), which results in induction of the cytochrome P450 and other enzyme systems. The polynuclear aromatic hydrocarbons (PAHs) are another class of omnipresent environmental contaminants that can bind to the Ah receptor.

Experimental studies have demonstrated that contaminants such as PHAHs and PAHs have the ability to alter thyroid hormone levels, thyroid gland structure, and vitamin A levels through a variety of mechanisms (reviewed in Zile, 1992; Brouwer et al., 1998). It has been well established that PHAHs can disrupt vitamin A metabolism, depleting body stores (Zile, 1992; Spear et al., 1986). In mammals and birds, both thyroxine (T_4) and retinol (the main circulating form of vitamin A) are transported in the blood on the same carrier protein complex, transthyretin (Goodman, 1980). Hydroxylated metabolites of some PCB congeners can interfere with transthyretin, resulting in decreased circulating levels of thyroxine and retinol (Brouwer et al., 1986; Brouwer and van den Berg, 1986; Brouwer et al., 1988; Brouwer et al., 1990). PHAHs are also known to interfere with the enzymes controlling thyroid hormone metabolism (Brouwer et al., 1998).

Because of the documented effects of PHAHs on both retinoids and thyroid hormones, these physiological parameters have been suggested as sensitive biomarkers of exposure to these chemicals in the environment (Peakall, 1992; Fox, 1993). Levels of thyroid hormones and retinoids can be influenced by many other factors including age, gender, diet, nutritional status, season and physiological condition. Therefore, studies should be designed to control for these variables and results should be interpreted accordingly.

Vitamin A (retinoids) and thyroid hormones play critical roles during development, growth and function throughout life. Retinoic acid (RA) is believed to have endocrine-like properties based upon the recognition that its nuclear receptors are structurally homologous to those of steroid and thyroid hormones (Giguère et al., 1987; Petkovich et al., 1987). Vitamin A is essential to normal differentiation of epithelial structures, reproduction, vision and immune system function (Zile, 1983). A gradient of RA in limb buds controls normal limb development in avian and mammalian embryos (Thaller and Eichele, 1987), and excessive or insufficient amounts can result in malformations. Thyroid hormones are involved in vertebrate development, metamorphosis in amphibians and some fish (e.g., flounder, eels) as well as growth and regulation of metabolic processes (McNabb and King, 1993). Alterations in thyroid function are known to affect mammalian reproduction (Leathem, 1972).

In this paper I review 22 field studies of wildlife and fish that report thyroid and/or vitamin A alterations associated with exposure to persistent synthetic chemicals in the environment. I also discuss relevant experimental studies that support the field observations, and shed light on the mechanisms of action involved. Adverse health effects reported in wildlife and fish in the field studies are discussed, but because ex-

perimental studies proving the mechanistic links are lacking, associations between these effects and retinoid and thyroid alterations are speculative. The goal of this review is to summarize the changes in thyroid gland structure, function and vitamin A status possibly associated with exposure to PHAHs and PAHs in several species of wildlife and fish. I examine the use of these parameters as biomarkers of exposure to these environmental chemicals, and suggest that some adverse health effects in wildlife and fish may be associated with alterations in thyroid and vitamin A status. Information about marine mammals will be described first, followed by avian species and fish.

MARINE MAMMALS

Field studies that have reported alterations in thyroid gland morphology, thyroid hormones, and retinoid levels in marine mammals associated with exposure to PHAH's are summarized in Table 1.

Thyroid gland histological changes

Morphological changes in the thyroid gland associated with an elevated tissue burden of persistent organochlorine chemicals (OCs) have been reported in harbor seals (*Phoca vitulina*) and in beluga whales (*Delphinapterus leucas*). Histological examination of the thyroid glands from 40 harbor seals that died in the North Sea during the 1988–89 epizootic of phocine distemper virus revealed colloid depletion and interfollicular fibrosis in most of the seals (Schumacher et al., 1993). Seals that died during this epizootic had higher tissue levels of OCs compared to survivors (Hall et al., 1992), especially the PCBs (Luckas et al., 1990). No thyroid gland colloid depletion or fibrosis was seen in healthy control seals from Iceland that had much lower levels of OCs and were negative for antibodies to canine distemper virus and phocine distemper virus. Similar but less severe changes were seen in the thyroid glands of seven harbor porpoises (*Phocoena phocoena*) from the North Sea. The porpoises inhabit the same region as the seals but were unaffected by the viral epizootic. The fibrosis seen in both types of marine mammals from the North Sea was characterized by the presence of mature collagen fibrils and the absence of vascularization, suggesting that these changes were due to a chronic process, and probably not associated with an acute event such as the viral epizootic.

Morphological changes in the thyroid gland have also been reported in beluga whales inhabiting the St. Lawrence estuary (De Guise et al., 1995). This is an endangered population that has very elevated levels of organochlorine pollutants and experiences a high incidence of neoplastic lesions, secondary bacterial infections and other pathologic lesions, along with a reduced rate of reproduction (Béland et al., 1993). Morphological changes observed during postmortem examination have included thyroid abscesses and one case of a thyroid adenoma.

Thyroid hormone and retinoid alterations

Investigators have hypothesized that PCB exposure accompanied by depressed levels of thyroid hormones and retinol may play a role in the pathogenesis of a skin disease affecting stranded immature northern elephant seals (*Mirounga angustirostris*). This condition, named northern elephant seal skin disease, is a generalized ulcerative dermatitis characterized histologically by hyperkeratosis of surface and follicular epithelium, acanthosis, and squamous metaplasia and atrophy of sebaceous glands. Affected seals have depressed levels of total triidothyronine (TT_3), total thyroxine (TT_4) and retinol, and elevated serum levels of total PCB's and dichloro-diphenyl-dichloroethylene (p, p′-DDE) compared to unaffected controls (Beckman et al., 1997).

Exposure to PHAH's (PCBs and polybrominated biphenyls) has been linked with hyperkeratosis accompanied by squamous metaplasia of sebaceous glands in humans, cattle, rodents, rabbits and nonhuman primates (Zinkl, 1977; Klein-Szan-

TABLE 1. Alterations in thyroid gland morphology, thyroid hormones and retinoid levels in marine mammals associated with exposure to polyhalogenated aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thyroid/retinoid changes	References
Harbor seal[a] (*Phoca vitulina*) Harbor porpoise (*Phocoena phocoena*)	North Sea	PCBs[b]	thyroid gland	thyroid colloid depletion interfollicular fibrosis	Schumacher et al., (1993)
Beluga whale (*Delphinapterus leucas*)	St. Lawrence Estuary, Quebec	PCBs other OCs[c]	thyroid gland	thyroid abscesses thyroid adenoma	De Guise et al., (1995)
Northern elephant seal (*Mirounga angustirostris*)	California	PCBs p,p′-DDE[d]	plasma	↓ retinol ↓ TT_4[e], TT_3[f]	Beckmen et al., (1997)
Harbor seal (*Phoca vitulina*)	captive	PCBs p,p′-DDE	plasma	↓ retinol ↓ TT_4, FT_4[g], TT_3	Brouwer et al., (1989)
Harbor seal (*Phoca vitulina*)	captive	PCBs dioxin TEQ's[h]	plasma	↓ retinol ↓ TT_4, TT_3[i]	De Swart et al., (1994, 1995)
Grey seal[j] (*Halichoerus grypus*)	Norway	PCBs	plasma	↓ TT_4, FT_4	Jenssen et al., (1995)
Grey seal[k] (*Halichoerus grypus*)	United Kingdom	PCB 169	plasma	↓ TT_3 : TT_4	Hall et al., (1998)

[a] Phocine distemper virus victims.
[b] Polychlorinated biphenyls.
[c] Organochlorine chemicals.
[d] Dichloro-diphenyl-dichloroethylene.
[e] Total thyroxine.
[f] Total triiodothyronine.
[g] Free thyroxine.
[h] Dioxin toxic equivalents.
[i] During fasting.
[j] Pups only.
[k] Pups only.

to et al., 1991; Jubb et al., 1993). Squamous metaplasia of sebaceous glands is pathognomonic of PHAH exposure in humans (Crow, 1991). However, squamous metaplasia of meibomian glands is a feature of PCB toxicosis that was not seen in the elephant seals (Beckman et al., 1997). An ulcerative dermatitis was also described in grey seals in the Baltic Sea and the investigators hypothesized that it may be associated with the elevated levels of PCBs in that ecosystem (Bergman and Olsson, 1985).

Thyroid hormones are important to maintaining skin integrity, but the skin lesions in the northern elephant seals were not typical of those seen in hypothyroid dermatopathies in other species (Scott, 1982). Skin lesions similar to those seen with vitamin A deficiency have been described with PHAH toxicity (Zile, 1992). Hyperkeratotic skin lesions are a feature of vitamin A deficiency in mammals, but squamous metaplasia of sebaceous glands has not been described with his condition (Pitt, 1985; Jubb et al., 1993). Therefore, PCB exposure could potentially underlie the thyroid and retinol alterations and the occurrence of this skin disease in the northern elephant seals. However, whether the depressed retinol and thyroid levels contributed directly to the etiology of this disease is unclear.

Two experimental studies using captive harbor seals have found a relationship between consumption of contaminated fish and reductions in plasma thyroid hormones and retinol, accompanied by altered reproductive function or immune system changes. In one 2-yr study, a group of 12 female seals was fed a diet of fish (mostly plaice, flounder and dab) from the polluted western Wadden Sea while the reference group was fed fish (mainly mackerel) from the relatively uncontaminated northeast Atlantic Ocean (Reijnders, 1986). The fish from the Wadden Sea were significantly more contaminated with PCBs and p, p′-DDE compared to the control diet. Three males fed the reference group diet were rotated between the two female groups allowing mating to occur throughout the study. There was a significant decrease in pregnancy in the females fed the more contaminated diet over the 2-yr period (Reijnders, 1986). This appeared to be caused by disruption of implantation caused by a smaller rise in levels of estradiol in the group fed contaminated fish, although this association could not be statistically tested because there were only two non-pregnant animals in the control group. The decreased reproductive success in seals fed the contaminated fish is particularly noteworthy because of the drastic reduction in the seal population in the 1960s and 1970s in the western part of the Wadden Sea apparently resulting from decreased pup production (Reijnders, 1978).

Both pregnant and non-pregnant seals on the more contaminated diet had significantly lower plasma levels of retinol (30–55%) at two sampling intervals during the study. Reductions in TT_3, TT_4 and free thyroxine (FT_4) occurred at one sampling interval, but were less dramatically decreased compared to retinol levels (Brouwer et al., 1989). The reductions in plasma retinol normalized 6 mo after the seals were switched to the control group diet. Based upon supporting evidence from experimental studies, the authors concluded that the decreased plasma retinol and thyroid hormones were most likely the result of PCB exposure. Whether the reproductive effects were directly related to the thyroid or retinoid alterations, or independent consequences of contaminant exposure is unclear from this study.

A second study examined immune system changes in juvenile captive harbor seals fed contaminated fish from the Baltic Sea (De Swart et al., 1994). Age and gender matched seals were fed either contaminated herring from the Baltic Sea or herring from Atlantic Ocean for 93 wk, and blood samples were obtained every 6 to 9 wk. Concentrations of OCs in the extractable fat of the fish were measured, includ-

ing congener specific analysis of PCDDs, PCDFs and coplanar PCBs. The average daily intake of the Ah-receptor-binding OCs in toxic equivalents (TEQs) relative to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (2, 3, 7, 8-TCDD or dioxin) was 288 ng TEQ/day in seals fed Baltic fish compared to 29 ng TEQ/day in the reference group. PCBs accounted for 93% of the TEQs. Blubber biopsies taken at wk 104 found 208.7 ± 11.6 ng TEQ/kg lipid in the seals fed Baltic fish compared to 61.8 ± 4.13 ng TEQ/kg lipid in the reference group. Several parameters of basal immune function were significantly suppressed including natural killer cell activity and specific T-cell responses. Circulating vitamin A levels were depressed throughout the study in seals fed the Baltic fish, similar to the findings in the study of seals fed Wadden Sea fish described previously.

Additionally, in a short-term study, both experimental groups underwent a 15-day fast in order to study mobilization of OCs from blubber. While the reference group showed an increase in circulating TT_3 and TT_4 during the fast, the group fed Baltic fish had significantly lower plasma TT_3 and TT_4 over the course of the fast (De Swart et al., 1995). When laboratory rats were put on the same fish diets, the group fed Baltic fish had significantly depressed plasma TT_4 levels (Ross, 1995). Increased levels of thyroid hormones may be part of the physiologic response to a fasting state in seals, a time during which lipids from blubber are utilized as the primary energy source. These results suggest that the Baltic fish-fed seals may be less able to adapt during fasting.

Studies of free-ranging seals have been more equivocal. A study of wild Norwegian grey seal pups (*Halichoerus grypus*) found that the ratio of TT_4:FT_4 was negatively correlated to serum concentrations of PCBs (Jenssen et al., 1995). However, this study did not control for the effects of age on circulating T_4 levels. Another study looking at the relationship between thyroid hormones and PCB exposure in young grey seals in the United Kingdom found declining T_4 levels with age but no relationship between blubber concentrations of PCBs and the ratio of TT_4:FT_4 (Hall et al., 1998). However, this study did find a significant relationship between the concentration of PCB 169 (3, 3′, 4, 4′, 5, 5′-hexachlorobiphenyl) and the ratio of TT_3:TT_4 when stage of lactation was used as a covariate (no relationship was seen for adult seals). As this study demonstrates, interpretation of thyroid hormone alterations must include a consideration of age and other variables such as nutritional status and physiological state that are known to be associated with natural hormonal variations.

AVIAN SPECIES

Field studies reporting alterations in thyroid gland morphology, thyroid hormones and retinoid levels in avian species associated with exposure to PHAH's are summarized in Table 2.

Thyroid gland histological changes

Histological abnormalities of the thyroid gland have been reported in herring gulls (*Larus argentatus*) nesting around the Laurentian Great Lakes. Thyroid gland histology and morphology were examined in 213 herring gulls collected from nine colonies during the period 1974–83 (Moccia et al., 1986). Reference thyroid glands were collected from a colony of herring gulls in the Bay of Fundy (1977–82), an iodine rich marine environment with lower concentrations of PHAHs. Compared to the reference population, the majority of Great Lakes gulls had an increased thyroid mass and diffuse microfollicular hyperplasia of the thyroid gland. The severity of the lesions varied both between and within lakes, and the spatial and temporal distribution of the severity of the thyroid abnormalities supported the hypothesis that the changes may be related to PHAH exposure.

A more recent study analyzed the hepatic OC residues (PCBs, DDE, dieldrin

and Mirex) and several biomarkers including thyroid mass in eight Great Lakes herring gull colonies and an Atlantic coast reference colony from 1974–93 (Fox et al., 1998). The OC concentrations decreased at most sites over the study period, with the greatest declines occurring prior to 1985. A concomitant decrease in thyroid gland enlargement (from moderate to slight) was consistent with the decline in OC levels.

Although the Great Lakes basin is an endemic region of iodine deficiency goiter in humans, the observations from these studies and others are not consistent with an iodine deficiency etiology as the sole cause of the lesions observed in the herring gulls. The temporal and spatial occurrence of thyroid pathology seen in the herring gulls does not correlate with inter-lake differences in iodine content (Moccia et al., 1986). Rather, the evidence suggests that a thyrotoxic factor exists in the Great Lakes food web and lipophilic OCs have been studied as possible causative agents. Laboratory studies showing thyroid dysfunction in rats fed Great Lakes coho salmon (*Oncorhynchus kisutch*) support the concept that Great Lakes fish carry a goitrogenic factor (Sonstegard and Leatherland, 1979; Villeneuve et al., 1981), which is the most likely exposure route for fish-eating Great Lakes gulls.

Experimental studies with several avian species support the hypothesis that exposure to PCBs and other persistent OCs can induce histological changes in the avian thyroid gland. Pigeons (*Columba livia*) dosed with p, p′-dichloro-diphenyl-trichloroethane (p, p′-DDT), p, p′-DDE and dieldrin had histological changes in the thyroid gland consisting of hypertrophy, hyperplasia and colloid depletion (Jefferies and French, 1969, 1972). Lesser black-backed gulls (*Larus fuscus*) dosed with Arochlor 1254 (a commercial PCB mixture) had increased thyroid weight and size, with histological changes resembling thyroid goiter (Jefferies and Parslow, 1972). No increase in thyroid weight was seen with increased dosage rates in this study, and the biggest thyroids were seen with the two lowest dosages (50 and 100 mg/kg/day). Guillemots (*Uria aalge*) dosed with Arochlor 1254 showed an increase in thyroid size, colloid area, and follicle size at lower doses (12 and 25 mg/kg/day) and progressive dose-related decreases in these parameters at higher doses (up to 400 mg/kg/day) (Jefferies and Parslow, 1976). The weight of the anterior lobe of the pituitary gland also decreased in a dose-related manner, suggesting that higher levels of PCBs had a direct effect on the pituitary causing a dose-related decrease in thyrotropin leading to secondary thyroid gland atrophy. The concentration of PCBs in the liver of the experimental guillemots was within the range of levels measured in wild guillemots from the North Atlantic.

Thyroid hormone and retinoid alterations

Liver levels of retinyl palmitate (the esterified storage form of vitamin A) were measured in 130 Great Lakes herring gulls and in gulls from two Atlantic coastal colonies during 1980–85 (Government of Canada, 1991). Generally marine gulls have a high dietary availability of retinoids and vitamin A precursor carotenoids making them a good reference population. Overall, hepatic retinyl palmitate levels from the Atlantic coastal gulls were significantly higher than those from Great Lakes gulls. While some Great Lakes colonies had retinoid levels similar to the reference population (possibly due to compensation by adequate dietary vitamin A sources), other colonies showed severe depletion of liver retinyl palmitate. It is noteworthy that gulls from western Lake Erie had the most severe depletion of retinoids, and gulls from the same area had the most severe histological changes in the thyroid gland (Moccia et al., 1986).

A similar study also found that concentrations of hepatic retinol and retinyl palmitate were significantly lower in Great Lakes gulls as compared to New Brunswick (Canada) gulls, and there were sig-

TABLE 2. Alterations in thyroid gland morphology, thyroid hormones and retinoid levels in free-ranging avian species associated with exposure to polyhalogenated aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thryoid/retinoid changes	References
Herring gulls (*Larus argentatus*)	Great Lakes	PHAHs[a]	thyroid	↑ thyroid mass thyroid hyperplasia	Moccia et al., (1986)
Herring gulls (*Larus argentatus*)	Great Lakes	PHAHs	liver	↓ retinyl palmitate	Government of Canada, (1991)
Herring gulls (*Larus argentatus*)	Great Lakes	2,3,7,8-TCDD[b]	liver	↓ retinol ↓ retinyl palmitate	Spear et al., (1986, 1992)
Herring gulls (*Larus argentatus*)	Lakes Huron, Erie, Ontario	2,3,7,8-TCDD ΣPCDDs + PCDFs[c] dioxin TEQs[d]	egg yolk	↑ retinol : retinyl palmitate	Spear et al., (1990)
Great blue herons (*Area herodias*)	St. Lawrence River, Quebec	ΣPCBs 105 + 118[e] ΣPCBs 105 + 118 TEQs[f]	egg yolk	↓ retinyl palmitate	Boily et al., (1994)
Cormorants (*Phalacrocorax carbo*)	Netherlands	PCBs[g] PCDDs PCDFs	egg yolk liver plasma	↓ FT_4[h] ↑ EROD[i]	van den Berg et al., (1994)
Herring gulls (*Larus argentatus*) Caspian terns (*Sterna caspia*)	Great Lakes	PCBs[j]	plasma[k]	↓ retinol	Grasman et al., (1996)
Common terns (*Sterna hirundo*)	Belgium Netherlands	mono-ortho PCBs PCDDs PCDFs	egg yolk plasma liver	↓ retinyl palmitate[l] ↓ TT_3[m], TT_4[n], FT_4 ↑ plasma retinol to yolk sac retinyl palmitate	Murk et al., (1996)
Herring gulls (*Larus argentatus*)	Great Lakes	PCBs, DDE,[o] dieldrin, mirex	liver	↓ retinyl palmitate	Fox et al., (1998)
Tree swallows[p] (*Tachycineta bicolor*)	Great Lakes St. Lawrence River basin	Ah-inducing chemicals[q]	liver	↓ retinol ↑ EROD	Bishop et al., (1999)

nificant differences among the Great Lakes colonies (Spear et al., 1986). Retinoid levels in both the coastal and Great Lakes colonies were inversely proportional to dioxin levels reported at these sites. Among the Great Lakes colonies, Lake Ontario gulls had the lowest retinoid levels and the highest dioxin contamination while Lake Superior gulls had the highest retinoids and lowest dioxin levels. Experimental studies using a single intraperitoneal injection of the dioxin-like PCB 77 (3, 3′, 4, 4′-tetrachlorobiphenyl) in ring doves (*Streptopelia risoria*) found that retinol levels significantly decreased as aryl hydrocarbon hydroxylase activity increased (Spear et al., 1986), supporting the relationship seen in the wild gulls between dioxin and retinoid status.

Fox et al. (1998) compared levels of hepatic retinyl palmitate and hepatic OC residues (PCB, DDE, dieldrin and mirex) in herring gulls from eight Great Lakes colonies and a coastal reference colony from 1974–93. The concentrations of most contaminants decreased over time, particularly prior to 1985. Retinyl palmitate levels increased significantly at two colonies over the same period, but despite the lower levels of OCs, moderate to severe depletion of vitamin A stores persisted at some sites. This could be related to changes in the dietary availability of vitamin A and/or continuing effects of contaminants on the ability to store vitamin A.

Grasman et al. (1996) found diminished T-cell immune system responses and lower plasma retinol levels associated with higher exposure to OCs (primarily PCBs) in prefledgling herring gulls and Caspian terns (*Sterna caspia*) exposed to a gradient of pollution in the Great Lakes. In both species, there was a negative relationship between total PCBs in eggs from the colony (reflecting perinatal exposure) and plasma retinol in chicks. No significant association between thyroxine levels and OC exposure was seen, and there was no apparent relationship between the lower levels of plasma retinol and the altered immune system parameters. This study did not control for variability in dietary availability of vitamin A precursors between colonies, so it is not possible to determine whether the decreased plasma retinol was directly related to PCB exposure, the result of variations in dietary availability of vitamin A precursors, or a combination of factors.

Egg yolk is a reservoir of retinoids available to avian embryos during development. Retinoids from the maternal circulation and body stores are incorporated into the lipid-rich egg yolk, primarily as retinol and retinyl palmitate. Measurement of retinoid concentrations in egg yolk

←

[a] Polyhalogenated aromatic hydrocarbons.
[b] 2,3,7,8-tetrachlorodibenzo-p-dioxin.
[c] Sum of the concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans.
[d] Dioxin toxic equivalents.
[e] Sum of the concentrations of polychlorinated biphenyls 105 and 118.
[f] Sum of polychlorinated biphenyls 105 and 108 toxic equivalents.
[g] Present in the highest concentrations—believed responsible for the effects seen.
[h] Free thyroxine.
[i] Ethoxyresorufin-O-deethylase.
[j] In egg yolk.
[k] In prefledgling chicks.
[l] In egg yolk.
[m] Total triiodothyronine.
[n] Total thyroxine.
[o] Dichloro-diphenyl-dichloroethylene.
[p] 16-day-old hatchlings.
[q] Aryl hydrocarbon receptor-binding chemicals. Decreases in retinol were correlated with EROD induction.

and the molar ratio of retinol to retinyl palmitate in egg yolk have been used to characterize the vitamin A status of developing avian embryos exposed to various levels of contaminants in the environment (Spear et al., 1990). Embryos utilize retinoids in the later stages of incubation, therefore the timing of sampling becomes important. In herring gulls the concentration of retinol and the molar ratio of retinol to retinyl palmitate are stable during days two to twelve of incubation, but decline thereafter because of utilization of retinol by the developing embryo (Spear et al., 1990). Therefore, it is optimal to collect the eggs during these early stages of incubation when the retinol levels are normally more stable.

Polychlorinated dibenzofurans, PCDDs and retinoids were measured in herring gull eggs collected during early incubation in 1986–87 from seven colonies in Lakes Huron, Ontario and Erie (Spear et al., 1990). The molar ratio of retinol to retinyl palmitate was positively correlated with the sum of the concentrations of the PCDDs and PCDFs measured at the various sites, with the concentration of 2, 3, 7, 8-TCDD (the major contaminant at most sites) and with the sum of the PCDD and PCDF toxic equivalents. No correlation was found between contaminant concentrations and the individual levels of either retinol or retinyl palmitate.

Retinoid and β-carotene (retinoid precursor) levels in eggs from seven great blue heron (*Area herodias*) colonies along the St. Lawrence River and at two reference sites were compared along with the concentration of 17 organochlorine chemicals and 42 polychlorinated biphenyl congeners in eggs from the same clutch (Boily et al., 1994). While retinol and β-carotene levels did not vary significantly between sites, the molar ratio of retinol to retinyl palmitate and the concentration of retinyl palmitate in eggs did vary significantly between colonies. Significant negative correlations were found between retinyl palmitate in eggs and the sum of PCBs 105 (2, 3, 3′, 4, 4′-pentachlorobiphenyl) and 118 (2, 3′, 4, 4′, 5-pentachlorobiphenyl) and TCDD-EQs based on PCBs 105 and 118 (mono-ortho coplanar congeners). Negative correlations were also observed with three mono-ortho-tetrachlorobiphenyls that are usually not considered environmentally relevant. The lack of variation between colonies in the levels of β-carotene and retinol implies that a dietary deficiency probably did not contribute to the lower retinyl palmitate levels in eggs from the more contaminated colonies.

A study of tree swallows (*Tachycineta bicolor*) at seven sites around the Great Lakes and the St. Lawrence River found no association between PCBs and retinoid levels in nestling liver or eggs (Bishop et al., 1999). However, liver EROD (ethoxyresorufin-O-deethylase) induction was negatively correlated with nestling liver retinol levels. EROD activity is a specific and sensitive biomarker of exposure to TCDD and related chemicals acting through the Ah receptor in avian species (Kubiak et al., 1989). Diet samples were analyzed for retinol and α- and β-carotene and there was no indication of limited vitamin A availability. Because induction of EROD is generally associated with exposure to coplanar PCBs, other dioxin-like contaminants or PAHs, these results imply that the decreased hepatic retinol was associated with exposure to Ah-inducing chemicals. The authors found no significant decreases in hatching or fledging success at any of these sites indicating that although hepatic retinol levels were depressed in nestlings, sufficient retinoids were available from dietary sources or through compensatory mechanisms to allow for apparently normal reproductive success.

The relationship between exposure to PCDDs and PCDFs, and alterations in retinoids, plasma thyroid hormone levels, and breeding parameters was examined in a study of eight common tern colonies (*Sterna hirundo*) in Belgium and the Netherlands exposed to a gradient of pol-

lution levels (Murk et al., 1996). Data on the colonies was grouped based upon yolk sac PCDD/PCDF, and associations with time of egg laying, incubation time and size of eggs and chicks were examined. Unfavorable breeding parameters (later egg laying, prolonged incubation and smaller eggs and chicks) were correlated with higher yolk sac PHAH concentrations, hepatic cytochrome P4501A1 induction, decreased yolk sac retinoids and plasma thyroid hormone levels in hatchlings, and an increase in the ratio of plasma retinol in hatchlings to yolk sac retinyl palmitate. Again, dietary vitamin A availability was not controlled in this study, making it difficult to interpret the retinoid alterations.

Further evidence for contaminant-related impacts on reproductive success and alterations in retinoid and thyroid hormone levels of hatchlings was found in another study in the Netherlands. This study examined cormorant (*Phalacrocorax carbo*) hatchlings from two colonies with different levels of exposure to PCBs, PCDDs and PCDFs (van den Berg et al., 1994). A 50% difference in reproductive success was observed between the two colonies in the field, and the more contaminated colony had two to five times the level of PCBs and 25% more PCDD and PCDFs in yolk sacs. Hatchlings from the more contaminated colony had increased EROD activity, a 50% decrease in plasma free T4, and an increased *in ovo* respiration rate. On an individual basis, significant concentration-response relationships were seen between EROD induction, plasma free T_4 reductions, yolk sac weight, liver weight, cranial size and concentrations of mono-ortho PCBs and/or PCDDs and PCDFs in the yolk sac. The weight at birth of hatchlings from the more contaminated colony was reduced, and the authors hypothesized that the weight reduction coupled with the increased in ovo respiration rate might indicate PCB-induced wasting disease which has been reported in other avian species (Kubiak et al., 1989).

Experimentally, the relationship between thyroid hormone alterations and exposure to Ah receptor-inducing contaminants has been inconsistent with the findings in field studies. Single dose exposure of adult great blue herons to 2, 3, 7, 8-TCDD resulted in a six-fold induction of EROD, significant elevation of plasma T_4 levels and no effect on plasma total T_3 or the plasma T_3:T_4 ratio (Janz and Bellward, 1997). In ovo exposure to low levels of 2, 3, 7, 8-TCDD failed to produce abnormalities in thyroid hormones in hatchlings of three species: the domestic chicken (*Gallus gallus*), domestic pigeon (*Gallus livia*), and the great blue heron (Janz and Bellward, 1996). Induction of hepatic microsomal EROD activity and decreased growth was observed in the pigeon and heron. In these studies, thyroid hormone responses to TCDD seem equivocal, although dosage, species sensitivity and varying experimental protocols all may influence the results. Thus, it is unclear whether the association between thyroid hormone alterations seen in some field studies are directly related to exposure to Ah-inducing chemicals, to other unmeasured contaminants, or to other biological factors.

Alterations in yolk retinoids have been demonstrated experimentally after PCB exposure. In a study designed to mimic environmental exposures, white leghorn hens (*Gallus domesticus*) were fed PCB's (0.5–6.6 mg/kg diet) derived from carp (*Cyprinus carpio*) from Saginaw Bay in the Great Lakes for a period of 7 wk (Zile et al., 1997). Retinoid levels were analyzed in 11-day-old embryos and eggs. Retinoid concentrations in the embryos were unaffected, however, the high PCB group showed a 50% reduction in the molar ratio of retinol to retinyl palmitate in eggs. In contrast, Spear et al. (1990) found a positive correlation between several indices of PCDD and PCDF concentrations and the molar ratio of retinol to retinyl palmitate in wild herring gull eggs. Many factors, including dietary availability of vitamin A, may be responsible for these different re-

sponses, but the experimental feeding study shows that prenatal exposure to PCBs can significantly affect the retinoid profile in eggs.

FISH

Field studies reporting alterations in thyroid gland morphology and retinoid levels in fish associated with exposure to PHAHs and PAHs are summarized in Table 3.

Thyroid gland histological changes

While thyroid enlargement (goiter) is rare in fish in the wild, the Pacific salmon introduced into the Great Lakes decades ago have experienced an epizootic of enlarged thyroid glands (Sonstegard and Leatherland, 1976). Every adult salmon examined from the mid-1970's to the early 1990's had evidence of thyroid gland hypertrophy and hyperplasia regardless of the lake of origin or the species when compared to comparable species and ages from the Pacific Northwest (reviewed in Leatherland, 1992, 1993). The lesions were similar histologically to those previously described in Great Lakes herring gulls (Moccia et al., 1986). Although there are inter-lake differences in levels of circulating thyroid hormones in affected salmon during gonadogenesis (Leatherland and Sonstegard 1981) and in eggs from maternal deposition (Leatherland et al., 1989), growth and development appear to proceed normally. Therefore, it appears that thyroid hormone levels in these salmon are still within the compensatory range of normal homeostasis.

As with the gulls, the available evidence argues against iodine deficiency as the cause of the thyroid enlargement in salmon (Leatherland and Sonstegard, 1984). In coho salmon there was no apparent correlation between lake or tissue iodide levels and the prevalence or size of goiters from different lakes, and experimentally it has not been possible to induce thyroid enlargement using iodide-free diets (Leatherland, 1993). Furthermore, levels of plasma thyroid hormones in coho salmon collected in the spring and summer months were high, which would not be possible with iodide deficiency (Leatherland, 1993).

As top predators in the Great Lakes food web, salmonids bioaccumulate significant levels of halogenated aromatic hydrocarbons, especially PCB's, and a role for some of these contaminants in the etiology of the thyroid enlargement has been investigated with equivocal results. No correlation between the severity of thyroid lesions in Great Lakes salmon and tissue levels of PCBs or mirex has been observed (Leatherland, 1992; Leatherland and Sonstegard, 1982). Experimentally, coho salmon and rainbow trout (*Oncorhynchus mykiss*) fed diets containing PCBs and mirex (chemicals found in Great Lakes salmon) failed to develop any thyroid enlargement (Leatherland and Sonstegard, 1978, 1979). Immature coho salmon fed diets composed of 100% Great Lakes salmon likewise failed to develop thyroid enlargement (Leatherland and Sonstegard, 1982). However, when laboratory rodents were fed diets containing 100% Great Lakes salmon, hypothyroidism, thyroid hyperplasia and goiters were seen in the rodents along with increased activity of cytochrome P450 enzymes and hepatomegaly (Sonstegard and Leatherland, 1979). The observed thyroid pathology correlated with the organochlorine content of the diet, and coupled with the changes in cytochrome P450 activity, these results suggest that these thyroid changes are associated with OCs found in Great Lakes salmon. The potential role of other environmental contaminants in the etiology of thyroid enlargement in Great Lakes salmon has not been investigated. These studies imply that Great Lakes salmon contain some substance that is goitrogenic for rodents, but it may not be the source of the goitrogenic factor affecting the salmon.

Although the direct cause of the thyroid enlargement in the Great Lakes salmon is not known, an environmentally-induced

TABLE 3. Alterations in thyroid gland morphology and retinoid levels in fish associated with exposure to polyhalogenated aromatic hydrocarbons and polynuclear aromatic hydrocarbons.

Species	Study location	Associated contaminants	Tissue sampled	Thyroid/retinoid changes	References
Salmon species (*Oncorhynchus* sp.)	Great Lakes	unknown factor	thyroid gland	thyroid hypertrophy, hyperplasia	Sonstegard et al., (1976)
White sucker (*Catostomus commersoni*)	Montreal, Quebec	coplanar PCBs[a]	liver	↓ retinol ↓ retinyl palmitate	Spear et al., (1992) Branchaud et al., (1995)
Lake sturgeon (*Acipenser fulvescens*)	Montreal, Quebec	PCBs	intestine	↓ retinyl palmitate ↓ dehydroretinyl palmitate	Ndayibagira et al., (1994)
Lake sturgeon (*Acipenser fulvescens*)	St. Lawrence River, Quebec	coplanar PCBs	liver	↑ RA[b] metabolism ↓ retinoids	Doyon et al., (1999)
Brown bullheads (*Ameiurus nebulosus*)	Great Lakes	PAHs[c]	liver	↓ retinyl palmitate ↓ dehydroretinyl palmitate	Arcand-Hoy et al., (1999)

[a] Polychlorinated biphenyls.
[b] Retinoic acid.
[c] Polynuclear aromatic hydrocarbons.

etiology appears likely. As mentioned earlier, thyroid gland enlargement has also been reported in herring gulls that feed on Great Lakes fish, supporting the hypothesis of a common environmental etiology (Moccia et al., 1981; Moccia et al., 1986). A re-evaluation of the status of thyroid enlargement in Great Lakes salmon and gulls along with current exposure levels to PCBs, mirex and other persistent contaminants of concern could shed light on the role that OCs have played in the etiology of this condition.

Experimentally, in an in vitro bioassay using slices of pig thyroid gland, water from Lake Erie inhibited synthesis of iodinated tyrosine and thyronine compounds indicating the presence of a goitrogenic substance in the water (Leatherland, 1993). It has been hypothesized that the goitrogenic factor may be a waterborne byproduct of bacterial metabolism, as such compounds have been shown to induce goiters in humans (Gaitán et al., 1980). Clearly, further research is needed to identify the goitrogenic substance(s) present in the Great Lakes food web.

Retinoid alterations

The scientific literature contains several field studies looking at the relationship between retinoid status and contaminant exposure in fish. An investigation into retinoid levels in larval white sucker (*Catostomus commersoni*) living in the contaminated Ottawa River near Montreal (Canada) was prompted by the observation of increased deformities in the adult population (Branchaud et al., 1995). Decreased liver retinoid stores had been previously documented in adult white sucker and in lake sturgeon (*Acipenser fulvescens*) captured near Montreal (Spear et al., 1992; Branchaud et al., 1995). Lower levels of intestinal retinoids (retinyl palmitate and dehydroretinyl palmitate) were also found in lake sturgeon captured near Montreal (Ndayibagira et al., 1994). When compared to a reference population at a relatively uncontaminated site on the same river, laboratory-raised larvae from fish collected at the Montreal site had very low hepatic stores of retinol and retinyl palmitate. Larvae from the contaminated site had an elevated incidence of deformities, and hepatic EROD activity in females was positively correlated with the occurrence of deformities in their progeny. These results suggest that compounds acting through the Ah receptor may be associated with the deformities. The principal Ah-active compounds present at this site were PCBs; the sum of coplanar PCBs in eggs from the affected fish was 36 times the level in fish from the reference site.

Doyon et al. (1998) documented up to a 40-fold lower level of hepatic retinoids in sturgeon from a site in the St. Lawrence River compared to a geographically separate reference population. Possible explanations included differences in food web-related dietary availability of vitamin A and exposure to PCBs in the St. Lawrence population.

Further studies of these sturgeon populations have examined the relationship between deformities, exposure to coplanar PCBs and RA metabolism (Doyon et al., 1999). Adult lake sturgeon from the St. Lawrence River had an estimated deformity rate of 2.9%, while larvae raised in artificial streams had a 6.3% rate of fin deformities, significantly higher than larvae from a reference site. To examine a possible relationship between the deformities and imbalances of RA, the cytochrome P450 dependant hydroxylation of RA to 4-hydroxy retinoic acid (4-OH-RA) and hepatic concentrations of retinoids and coplanar PCBs were measured. The rate of 4-OH-RA formation was over three-fold higher in the St. Lawrence sturgeon and the hepatic concentration of PCBs (expressed as toxic equivalents) was 20-fold higher compared to sturgeon from the reference site. Liver retinoid levels were negatively correlated with the RA hydroxylation rate. These results support an association between exposure to PCBs, increased metabolism of RA by cytochrome

P450 enzymes, lower hepatic retinoid stores, and an increase in developmental deformities in lake sturgeon.

Laboratory studies support the hypothesis that Ah receptor-active coplanar PCBs may be responsible for the altered retinoid status reported in fish. Exposure of adult brook trout (*Salvelinus fontinalis*) to a single intraperitoneal injection of PCB 77 decreased plasma retinol and intestinal retinoids in males (Ndayibagira et al., 1994). Oral exposure to the coplanar PCB 126 (3, 3′,4,4′,5-pentachlorobiphenyl) resulted in decreased liver retinoids in lake trout (*Salvelinus namaycush*) (Palace and Brown, 1994). In rainbow trout (*Onorhynchus mykiss*) exposure to PCB 77 increased RA hydroxylation through induction of cytochrome P4501A isoenzymes (Gilbert et al., 1995). Together these results suggest that increased retinoid metabolism induced by PCBs could cause the vitamin A depletion reported in the field studies.

Alterations in retinoids have also been associated with exposure to PAHs. In a study of brown bullheads (*Ameiurus nebulosus*) sampled from three PAH-contaminated sites in the lower Great Lakes, a significant correlation was found between induction of EROD activity and depleted hepatic retinoid stores (Arcand-Hoy and Metcalfe, 1999). Fish from the contaminated sites also had an increased hepatosomatic index and an elevated incidence of hepatic neoplasms compared to reference sites.

A relationship between PHAH and PAH exposure and alterations in plasma and hepatic retinols was also observed in an experimental mesocosm study in which flounder (*Platichthys flesus*) were exposed to polluted sludge from Rotterdam Harbor (The Netherlands) or sludge wash-off for 3 yr (Besselink et al., 1998). A significant decline was seen in plasma and hepatic retinols and hepatic retinyl palmitate stores in fish exposed to the sludge wash-off (but not to sludge alone). In this study, no relationship was found between induction of EROD activity and plasma or hepatic retinoid levels. Clearly more research is required to understand the role that Ah induction by planar PHAHs and PAHs plays in altering retinoid homeostasis in fish.

DISCUSSION

The studies reviewed in this paper have found associations between exposure to PHAHs or PAHs and histological changes of the thyroid gland, alterations in circulating thyroid hormone levels, and alterations in vitamin A homeostasis in a variety of wildlife and fish species (Tables 1, 2, 3). As this review demonstrates, similar observations have been made across both vertebrate classes and species. In some cases the observed changes in thyroid and/or vitamin A status were associated with adverse health effects, including decreased reproductive success and altered immune system function in harbor seals; skin disease in northern elephant seals; unfavorable breeding parameters and decreased reproductive success in common terns and cormorants, respectively; and developmental deformities in white suckers and lake sturgeon.

Experimental studies support the hypothesis that these observations in wildlife may be causally linked to exposure to some environmental contaminants. The PHAHs and PAHs are known to cause severe disturbances in vitamin A metabolism and homeostasis in laboratory species (reviewed in Zile, 1992). The known mechanisms of action include accelerated metabolism and breakdown of vitamin A and depletion from circulation and hepatic tissue stores. Certain coplanar PCBs have been shown to lower retinoid stores in laboratory rats (Spear et al., 1988), mice (Brouwer et al., 1985) and ring doves (Spear et al., 1986, 1989). Dioxin and related contaminants acting through the Ah receptor may interfere with vitamin A metabolism and storage through enzyme induction mechanisms because cytochrome P450 hydroxylation is a step in the retinoid metabolic pathway (Spear et al., 1992).

Numerous experimental studies have also documented disruption of circulating thyroid hormones and thyroid gland structure and function after exposure to dioxin, dioxin-like furans and PCBs, and hydroxylated metabolites of certain PCB congeners (reviewed in Brouwer et al., 1998). In addition to causing histological changes in the thyroid gland itself, PHAHs interfere with enzymes that regulate thyroid hormone metabolism and homeostasis including the UDP-glucuronyl-transferases that regulate T_4 glucuronidation and excretion; the iodothyronine deiodinases that activate and deactivate thyroid hormones; and the sulfotransferases that regulate free thyroid hormone concentrations in the blood (Brouwer et al., 1998). In addition, the hydroxylated metabolites of certain PCB congeners interfere with the plasma protein complex that transports both thyroxine and retinol in the blood resulting in decreased circulating levels of both substances (Brouwer and van den Berg, 1986; Brouwer et al., 1986, 1988, 1990).

Further research is required to determine if disruption of thyroid or vitamin A homeostasis is causally related to the adverse health effects reported in wildlife and fish exposed to PHAHs or PAHs. The health effects observed in the field studies reviewed in this paper could be the direct result of chemical toxicity. However, thyroid hormones and retinoids are essential to normal development, growth, epithelial integrity, immune function, reproduction and metabolism (Leathem, 1972; Zile, 1983; McNabb and King, 1993). Therefore, if thyroid or retinoid levels are altered beyond the limits of homeostatic compensation, adverse health effects may result. The consistency of responses seen among different vertebrate classes lends credence to an association that deserves further research. It has also been noted that some of the pathological effects of TCDD and TCDD-like chemicals in lab animals are quite similar to those caused by vitamin A deficiency (Thunberg, 1984). However, without knowledge of the underlying mechanisms involved to delineate a cause-effect relationship, any association between thyroid and retinoid alterations and the adverse health effects reported in these field studies is only circumstantial.

Alterations in thyroid hormones and vitamin A levels could prove to be useful in field studies as another biochemical biomarker of exposure to PHAHs and PAHs. Because of normal variations in thyroid hormones and retinoids associated with age, gender, diet, nutritional status, season and physiological condition, results must be interpreted with caution and studies should be designed to control for these variables. Researchers undertaking ecotoxicological investigations should consider including measurements of thyroid hormones and vitamin A levels and histological examination of thyroid gland structure when designing field research projects to study the responses to these chemicals in wildlife and fish populations.

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