Science of the Total Environment 408 (2010) 6117–6123

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Relationship between persistent halogenated organic contaminants and TCDD-toxic equivalents on EROD activity and retinoid and thyroid hormone status in northern fulmars

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ARTICLE INFO

Article history:
Received 11 June 2010
Received in revised form 24 August 2010
Accepted 26 August 2010

Keywords:
Arctic
Northern fulmar
Seabirds
Ethoxyresorufin-*O*-deethylase (EROD)
Thyroid hormones
Retinoids

ABSTRACT

We investigated whether the hepatic cytochrome P450 1A activity (measured as 7-ethoxyresorufin-*O*-deethylase (EROD)) and plasma thyroid hormone and liver retinoid concentrations were explained by liver and blood levels of halogenated organic contaminants (HOCs) in free-ranging breeding northern fulmars (*Fulmarus glacialis*) from Bjørnøya in the Norwegian Arctic. Hepatic EROD activity and liver levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalents (TEQs) were positively correlated, suggesting that hepatic EROD activity is a good indicator for dioxin and dioxin-like HOC exposure in breeding northern fulmars. There were not found other strong relationships between HOC concentrations and hepatic EROD activity, plasma thyroid or liver retinoid concentrations in the breeding northern fulmars. It is suggested that the HOC levels found in the breeding northern fulmars sampled on Bjørnøya were too low to affect plasma concentrations of thyroid hormones and liver levels of retinol and retinyl palmitate, and that hepatic EROD activity is a poor indicator of polychlorinated biphenyl (PCB) and pesticide exposure.

1. Introduction

Although the accumulation of halogenated organic compounds (HOCs) in seabirds is largely associated with diet (e.g. Borga et al., 2001), levels and patterns of HOCs may vary widely due to differences in form, content, substrate specificity and activity of xenobiotic-metabolizing enzymes comprised mainly within the cytochrome P450 (CYP) families 1 through 3 (Walker, 1990, 1998). Planar compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, other polychlorinated dibenzo-*p*-dioxins (PCDD), furans (PCDFs), non-*ortho* substituted polychlorinated biphenyls (PCBs) and mono-*ortho* substituted PCBs bind with various affinities to the cytosolic aryl hydrocarbon receptor. Binding of co-planar (dioxin-like) HOCs to the aryl hydrocarbon receptor induces the synthesis of CYP 1A enzymes, which are involved in the oxidative reaction of these compounds, as well as endogenous compounds (Parkinson, 2001). Induction of CYP 1A in birds has commonly been quantified as an increase in hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity (e.g. Elliott et al., 1996), although this biochemical response has been shown to be highly species-specific (Kennedy et al., 2003a).

The majority of toxicological effects following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or other dioxin-like compounds have been suggested to be mediated through binding to the aryl hydrocarbon receptor. The evidence for a common mechanism, and for additive effects, has led to the development of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxic equivalency factors and toxic equivalents (TEQs), by which the toxic potential of PCDD and dioxin-like aryl hydrocarbon receptor ligands can be quantified (Safe, 1990; Van den Berg et al., 1998, 2006). Among a range of effects, the aryl hydrocarbon receptor ligand formation may cause decreased storage and mobilization of thyroid hormones and retinoids (Brouwer et al., 1990, 1998; McNabb and Fox, 2003; Rolland, 2000). However, several other mechanisms are also proposed for HOC-induced thyroid hormone and retinoid disruption. Some HOC metabolites have a chemical configuration that is similar to those of thyroid hormones. Consequently, HOC metabolites may interact with transport proteins reducing the transport capacity of thyroid hormones in blood and cerebrospinal fluid (Letherland, 2000). If thyroxin (T4) is prevented by metabolites from occupying binding sites at the transport proteins, an increase in free T4 is expected, facilitating the excretion of free T4

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doi:10.1016/j.scitotenv.2010.08.037

(McNabb and Fox, 2003). HOCs can also increase the thyroid hormone uridine diphosphate glucoronosyl transferase. The conjugation product, T4-glucuronide, is readily excreted into the bile resulting in decreased thyroid hormone concentrations (McNabb and Fox, 2003). Furthermore, HOCs could disrupt the thyroid gland function and interfere with the iodothyronine deiodinases and sulfotransferases (Brouwer et al., 1998; Letherland, 2000; Rolland, 2000).

Hydroxy PCBs interfere with the formation of a retinol binding protein-transthyretin carrier complex. This in turn leads to an accelerated excretion of retinol binding protein via the kidney, lowered serum concentrations of retinol, and a diminished delivery of retinol to its target epithelia (Brouwer and van den Berg, 1986; Brouwer et al., 1986; Brouwer et al., 1990). Retinol is also lost through cytochrome P450 hydroxylation and uridine diphosphate glucuronyltransferase conjugations (Spear et al., 1988; Giesy et al., 2003). Additionally, enzymes involved in estrification of free retinol for storage may be inhibited by dioxin and dioxin-like HOCs affecting the uptake of retinyl esters from chylomicrons and the mobilization of stored retinyl esters (Ndayibaira and Spear, 1999).

Correlative studies have indicated reproductive, behavioural and immunological effects of HOC exposure in free-ranging Arctic glaucous gulls (*Larus hyperboreus*) (Verreault et al., 2010). Furthermore, HOC-induced hepatic EROD activity and/or disruption of the retinoid and thyroid hormone homeostasis has been reported in free-ranging juvenile and adult bald eagles (*Haliaeetus leucocephalus*) (Elliott et al., 1996), black guillemots (*Cepphus grylle*) (Kuzyk et al., 2003), European shags (*Phalacrocorax aristotelis*) (Murvoll et al., 1999) and glaucous gulls (Verreault et al., 2004).

Northern fulmars (*Fulmarus glacialis*) from the Norwegian Arctic (Bjørnøya) (Knudsen et al., 2007), the Canadian Arctic (Braune and Simon, 2003; Braune et al., accepted for publication; Fisk et al., 2001; Mallory et al., 2006, 2007) and the Faroe Islands (Fängström et al., 2005) have been reported to accumulate high levels of dioxins, furans, oxychlordane and mercury. There are no previous reports of effect studies in northern fulmars, except for a collaborate study carried out in Devon Island and Prince Leopold Island, Canada (Braune et al., submitted for publication). Based on the reports of high levels of dioxins, furans, oxychlordane and mercury, the northern fulmar is an interesting candidate for studying effects of HOCs in Arctic wildlife. The present study investigated whether hepatic EROD enzyme activity, retinoid and thyroid hormone concentrations in free-ranging breeding northern fulmars from Bjørnøya were related to blood and liver concentrations of HOCs (PCBs, pesticides, dioxins, furans) and/or TEQs (based on toxic equivalency factors for birds (Van Den Berg et al., 1998)). We predicted i) a positive relationship between hepatic EROD activity and dioxin-like HOCs, ii) an inverse relationship between concentrations of HOCs and total T4, iii) a positive relationship between HOCs and free T4, and iv) an inverse relationship between HOCs and hepatic concentrations of retinol and retinyl esters. It was difficult however to predict the relationship between triiodothyronine (T3) and HOCs, as previous studies have reported contrasting results. Some studies have found a positive relationship between T3 and HOCs (Peacall, 1992; Verreault et al., 2004), whereas other studies have reported no effect or even negative effect of HOCs on the T3 hormone levels (Peacall, 1992; Jantz and Bellward, 1997; Chiba et al., 2001).

2. Materials and methods

2.1. Study area and species

Bjørnøya (74.30°N 19.01°E) is situated in the western margin of the Barents Sea, halfway between mainland Norway and the Svalbard archipelago. The southern part of Bjørnøya is characterized by steep cliffs up to 400 m high where the most dense seabird colonies are found. Approximately 500,000 pairs of seabirds breed on Bjørnøya annually, including 15,000 pairs of northern fulmars (Mehlum and Gabrielsen, 1995). Northern fulmars are surface feeders that obtain their prey from the upper part of the water column. As such, northern fulmars mainly feed on fish (and fish offal from offshore fisheries), squid, copepods and amphipods. Marine carrion is occasionally included in their diet (Anker-Nilssen et al., 2000). The fulmar is a summer resident of Arctic areas and migrates to the northern boundaries of the Atlantic Ocean during winter(Anker-Nilssen et al., 2000).

2.2. Sampling

A total of 15 incubating northern fulmars (6 females and 9 males) were captured at the southern part of Bjørnøya in 2003. The birds were captured on their nests using a noose-pole and blood was collected from the humeral–radial–ulnar joint with a heparinized 20 mL-syringe and a 21-gauge needle. The blood was centrifuged (5000 rpm; 7 min), and the plasma was stored in a propane-driven freezer (−10 °C). Following blood sampling, the birds were euthanized and the liver (left lobe) was immediately collected, rinsed in a solution of $NaCl/H_2O$ (9 g/L) and stored in liquid nitrogen within 10 min after euthanization. Capture and sampling procedures were approved by the Governor of Svalbard (Longyearbyen, Norway).

2.3. EROD analysis

EROD activity was measured in liver microsomes in Environment Canada's biomarker laboratory at the National Wildlife Research Centre (Ottawa, Canada) following methods described by Kennedy and Jones (1994), with modifications by Trudeau and Maisonneuve (2003). The laboratory is accredited for this method according to the requirements of the Canadian Association for Environmental Analytical Laboratories (CAEAL). In brief, liver microsomes were prepared by differential centrifugation of the liver homogenates. The first centrifugation step at 9000 g removed the cell debris, nuclei and mitochondria. The resulting supernatant (S-9 fraction) was then centrifuged at 100,000 × *g* for 60 min and the microsomal fraction was obtained by re-suspending the pellet in a phosphate buffer. The measurement of EROD activity in liver microsomes was based on the formation of resorufin from 7-ethoxyresorufin, in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). The microsomes were incubated for 10 min after which the reaction was stopped by precipitating the microsomal proteins with acetonitrile containing fluorescamine. The latter reacted with the primary amino groups of the protein to produce a fluorescent compound that was measured at EX400/EM460. Protein quantification was done against a BSA standard curve. In the same reaction mixture, resorufin was measured at EX530/EM590 and quantified against a resorufin standard curve. The rate of enzyme activity was reported in pmol of resorufin formed per minute per mg of total proteins (pmol/min/mg proteins). A control sample (i.e. biological material tested at Environment Canada) was included at each plate. Analyses were done in triplicate and repeated if the results differed by more than 5% at normal levels and 15% at low levels. In addition, standards were verified spectrophotometrically against a standard curve consisting of five different concentrations in triplicate. Protein concentrations were determined separately for each well to compensate for any variation in the microsome volume added.

2.4. Thyroid hormone analyses

Plasma samples were analysed for thyroid hormones at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway. The analyses were based on commercially-available Count-a-Count® (Diagnostic Products Corporation, Los Angeles, CA, USA) human kits for total T4 (nmol/L), free T4 (pmol/L), total T3 (nmol/L)