STRATEGY FOR ASSESSING IMPACTS OF THYROID DISRUPTING CHEMICALS IN TELEOSTEANS

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Introduction

Thyroid toxicants are generally defined as compounds that alter circulating levels of thyroid hormone [1]. Nowadays there are around 116 environmental compounds that are suspected to disrupt thyroid function [2]. However, studies of thyroid disruption often incorporate an incomplete picture of the dynamic relationship within the HPT axis. These relationships are quite complex, and measurements of some of these parameters can be very difficult to obtain. Thus it is important to capture endpoints that are more indicative of thyroid disruption as well as reflective of adverse effects. There are currently no *in vitro* or *in vivo* assays that are sufficiently developed to warrant recommendation for use to efficiently screen chemicals for thyroid disruption. The current study examines the effect of subchronic exposure to a complex mixture of commercial Aroclor standards on thyroid hormone physiology in teleosteans and presents some available methods that can be used to measure thyroid hormones, measure their metabolism and assess the thyroid histological appearance.

Materials and methods

A total of 75 juvenile sea bass (*Dicentrarchus labrax*) weighting 13.2±2.8 g participated in this study. Fish were housed in 200-L tanks with a natural photoperiod. The water temperature was maintained at 15°C during the experiment. Aeration was set to maintain the water at 100% saturation. The water was continuously filtered through mechanical, charcoal and extensive biological filters before being recycled. The contaminant mixture was formulated to reflect the persistent organic pollution to which the European sea bass population could conceivably be exposed. The dose levels of the 7 ICES tracer congeners in the mixture reflect the observed concentration in common sea bass prey. Fish were randomly assigned to control, 0.3 ppm, 0.6 ppm, 1.0 ppm and 10.0 ppm treatment groups (n=15 in each case). Fish were fed by spiked food for 120 days. The daily feeding rate was equal to 2.0% of the mean weight of the fish, adjusted after each sampling period based on mean weight of the sub-sample fish that were sacrificed. Feed was presented by sprinkling at the surface of the water and was generally consumed by each group of fish within 1 min. Five fishes were sampled from each tank on days 40, 80 and 120. Fish were always sampled 24 h after the previous feeding. The subpharyngeal region was dissected and immersed in formalin fixative. Approximately 10 g skeletal muscle was excised caudally from the head, dorsal to the lateral line and anterior to the dorsal fin. Muscle and liver samples were frozen immediately on dry ice and later stored at -80 °C until analysis. The effectively accumulated PCB levels were analyzed by GC-MS. Muscular thyroid hormone concentration and the main metabolic pathways for thyroid hormones (deiodination, glucuronidation and sulfatation) were assessed.

Results and discussion:

The fish thyroid cascade can be broken down into the following three elements. First is the centrally controlled brain-pituitary-thyroid axis, which is primarily responsible for synthesis, storage and secretion of T4 and maintenance of T4 levels. The second element is the peripherally controlled availability of the active hormone T3 by enzymatic deiodination of the prohormone thyroxine, T4. The third phase is the receptor-mediated effects of T3 on target cells to regulate development, growth and reproduction.

Disruption mechanisms of thyroxin production and liberation

The observed effects were different depending whether the exposure was environmentally relevant or exceeding the environmental concentrations. In fish exposed to concentrations exceeding those encountered in the environment, a depression of muscular T3 and T4 levels was observed (Figure 1).

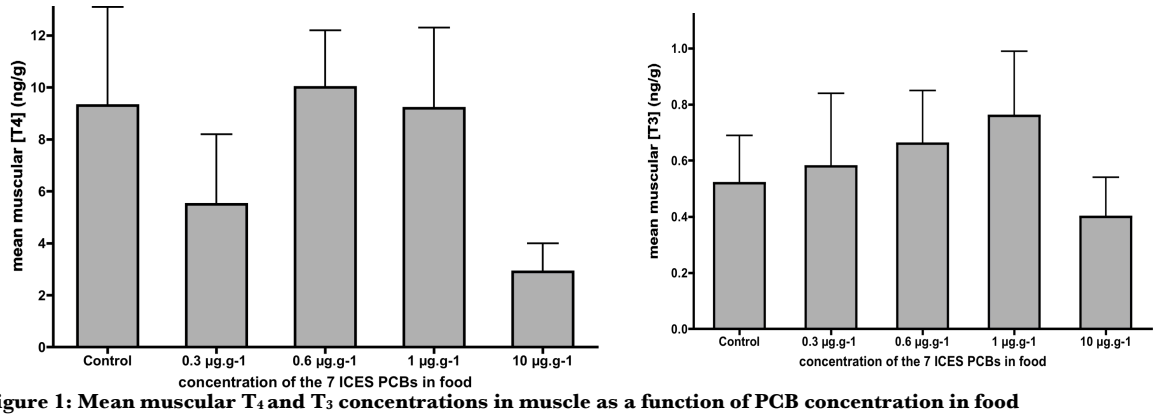

Figure 1: Mean muscular T_4 and T_3 concentrations in muscle as a function of PCB concentration in food

The centrally controlled thyroidal secretion of T4 was monitored adequately from the muscular T4 levels and from thyroid histological appearance. The size of the follicles and the form of the follicular cells gives an indication of the secretory activity of the gland. Thyroids dominated by small follicles lined by cuboid and columnar cells can be classified as highly active. Relatively inactive thyroids show large follicles lined by a flattened epithelium [3]. In fish exposed to environmentally relevant concentrations, muscular T4 levels were preserved and no multivariate relationship with contaminant exposure could be revealed. Measurements of follicular diameter and epithelial cell heights showed that higher contamination levels were capable of inducing a mild hypertrophy, indicating an increase of synthesis and secretion activity of the gland. There was a remarkable heterogeneity in how individual follicles responded to contaminant exposure [4]. Therefore, any minor proliferation of thyroid tissue is fairly difficult to determine.

Ultrastructurally, PCB-induced changes in thyroid gland include an increased development of endoplasmic reticulum and an accumulation of a large number of colloid droplets and large lysosomes (Figure 2). Similar observations have been made in PCB administered rats [5-7].

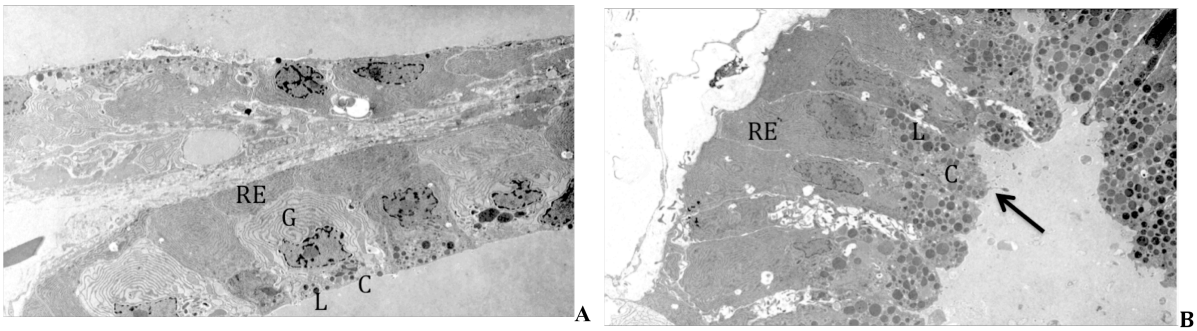

Figure 2 : Thyroid follicular cells of sea bass exposed to 1 µg g^{-1} Σ [7 PCBs] in food (x2000):

A between two smaller follicles, we can see few apical cytoplasmic processes extending into follicular lumen, well developed rough endoplasmic reticulum (RE) and large Golgi apparatuses (G) and few colloid droplets (C) and lysosomal bodies (L)

B. of large follicle, we can see apical cytoplasmic processes extending into follicular lumen (Arrow), dilated profiles of rough endoplasmic reticulum (RE) and compressed Golgi apparatuses (G) and numerous large colloid droplets (C) and lysosomal bodies (L)

Histological examinations of thyroids from animals exposed to 10 µg.g^{-1} dw [7 ICES PCBs] in food pellets revealed an enlargement of the interstitial tissue between follicles and degenerated colloid. The follicles appeared in lower numbers and the tissue seemed disorganized. These degenerative histological changes might have caused the hypothyroidism in these fish.

Thyroid hormone deiodination and metabolism

Peripheral T3 levels in teleost fish are largely controlled by enzymatic deiodinase activities in extra-thyroidal tissues. Exposure of rats to PCBs resulted in an inhibition of hepatic deiodinase activity [8-10]. In this study we observed an increase of T4-ORD related to contaminant exposure (Figure 3). Similar observations have been made in American plaice (*Hippoglossoides platessoides*) [11]. It was concluded that the PCB-induced changes in deiodinating activity likely represents compensatory responses to disrupting effects that might otherwise have depressed the plasma T3 levels.

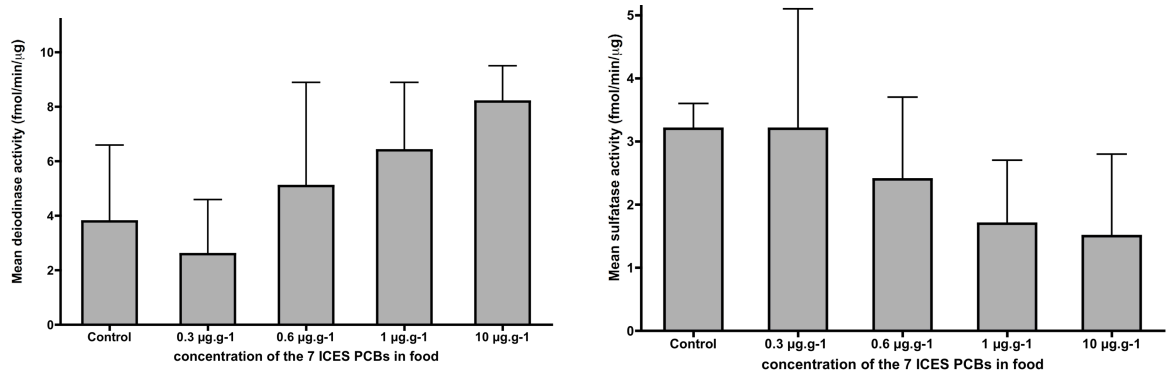

Figure 3 : Mean deiodinase and sulfation activity in sea bass liver as a function of PCB concentration in food

Additional pathways are important in metabolizing iodothyronines. These conjugation pathways include glucuronidation and sulfation of the phenolic hydroxyl group. Conjugation changes the solubility of iodothyronines, allowing their concentration in bile acids and excretion through the hepatic pathway. In general, there is a very large literature about the role of pollutants inducing glucuronidation, changing circulating levels of thyroid hormones [10,12-14]. Generally, phenolic PCBs undergo detoxification by glucuronidation and induce hepatic UGTs to facilitate excretion of PCBs [10,13]. We did not observe an increase in hepatic T4-UGT activity in our experimental exposure. This may be because the T4-glucuronidation enzyme assay is catalyzed by specific UGT forms other than those involved in the putative detoxification of Aroclor. Another step in the metabolism of iodothyronines is the sulfation by sulfotransferases [15]. In our experiment we observed a general decrease of SULT activity (Figure 4). This is in accordance with *in vitro* studies using rat and human hepatoma cell lines that related a strong inhibition of thyroid hormones sulfation by hydroxylated metabolites of PCB [8,15].

Thyroid hormone effects

The thyroid status has pronounced effects on growth and development in fish [16-19]. Depending on the dosage used, T_3 supplementation has anabolic and catabolic effects whereas hypothyroidism always results in growth retardation [20,21]. In this study, neither size, nor weight differences could be found between the treatment groups, though small differences in growth and specific growth rates could be observed. Xenobiotic-induced changes in thyroid hormone function have yet to be conclusively causally linked to decreased fitness or survival [17,22].

Conclusions

A recent review [17] failed to find a satisfactory assay for evaluation of biological responses that are unique to thyroid function. The attribution of xenobiotic effects to the thyroid function is extremely complex. Numerous variables must be taken into account to distinguish indirect and direct actions on the thyroid cascade from chemical exposure [22]. Assays for post-receptor biological actions of T_3 are difficult to develop in fish. Consideration should be given to early development of fishes that could become an interesting thyroid hormone effect screen. The use of model fish species like *Cyprinodon variegates* (marine species) or *Danio rerio*

(freshwater species) could be recommended. They are easy to maintain in the aquarium and are easily reproduced in captivity. Indeed, the early life stage may prove to be very susceptible to thyroid disruption. This necessarily requires a refinement of the assays presented here to apply to very small fish. Such an approach would allow characterizing developmental and transgenerational impacts of endocrine disrupting chemicals on fish larvae. Future projects should link different levels of analysis: endocrine at the thyroid system, protein expression patterns and epigenetics. Structural characterization of proteins of interest will allow a deep understanding of functional impairment induced by pollutants. Inheritance of reproductive abilities, of endocrine disruptions, and of a stress proteome could be tested on several generations of fish. Epigenetic mechanisms give potential explanations of the putative transgenerational impacts of pollutants.

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