The effects of di-n-butyl phthalate and 4-tert-octylphenol in osteoclastic and osteoblastic activities in teleost fish scales

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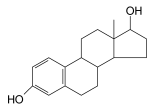
INTRODUCTION

Estrogenic endocrine disruptors are a diverse group of compounds with different chemical structures that have the capacity to interfere with the organism normal estrogenic actions.

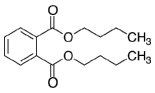
Di-n-butyl phthalate (DBP) is a phthalate commonly used as additive in plastics, paints, inks and cosmetics and 4-tert-octylphenol (OP) is an alkylphenol mainly used as emulsifier in detergents but also added to paints, pesticides, textiles and some personal care products. Both are widespread environmental pollutants with estrogenic activity. They have been shown to have endocrine disruptive actions in reproduction of several fish species and can bind fish estrogen receptors. However, their impact in bone and scale metabolism, which are estrogen-responsive tissues, remains unknown.

Like mammalian bone, fish scales are a dynamic tissue maintained by continuous cycles of formation and resorption mediated, respectively, by osteoclasts (OSC) and osteoblasts (OSB). They are an estrogen-responsive tissue, where the expression of nuclear estrogen receptors (ERs) have been detected. Moreover, tartrate-resistant acid phosphatase (TRAP) and alkaline phosphatase (ALP) enzymatic activities, used as markers for OSC and OSB activities, respectively, have been modified by estrogen in scales from several fish species.

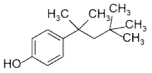
Using an in vitro bioassay, we investigated the possible impact of DBP and OP exposure on mineral metabolism in fish scales. The effects of these pollutants were assessed by determining TRAP and ALP activities of scales sampled from both a marine species (Atlantic sea bass, *Dicentrarchus labrax*) and a freshwater species (Mozambique tilapia, *Oreochromis mossambicus*).



Estradiol (E_2)



Di-n-butyl phthalate (DBP)



4-tert-octylphenol (OP)

METHODS

In vitro scale bioassay conditions:

Species: Sea bass (150-300g) and Mozambique tilapia (75-125g)

N= 4 scales / fish, 3 fish / treatment (dose and time)

Compounds tested: Di-n-butyl phthalate (DBP) and 4-tert-octylphenol (OP)

Concentration range: 10^{-6}, 10^{-8} or 10^{-10} M

Incubation time: 30 min and 24h

Enzymatic activities measured: TRAP (OSC marker); ALP (OSB marker)

Method: colorimetric method using p-nitrophenol (pNP) as substrate, quantification of the product p-nitrophenyl phosphate (pNPP) at 405nm

Fish scales sampling

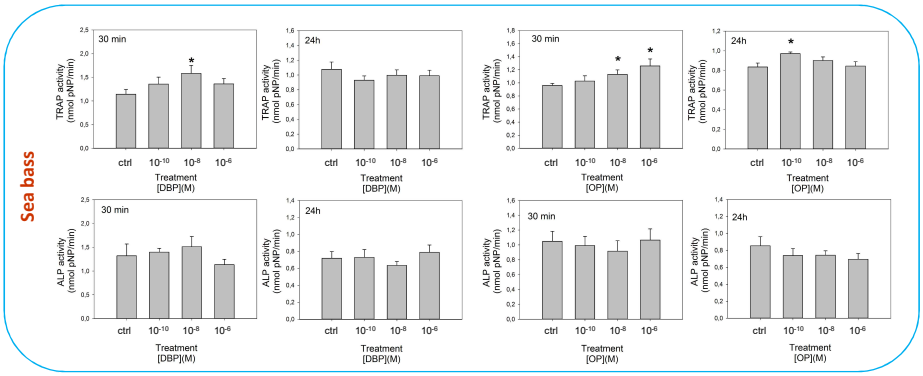
In vitro incubation

Colorimetric method for enzymatic activity (405 nm)

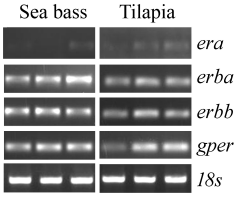
RESULTS

Di-n-butyl phtalate (DBP)

4-tert-octylphenol (OP)



Expression of estrogen receptors in scales



mRNA expression of nuclear estrogen receptors (era, erba and erbb) and of the membrane G protein-coupled estrogen receptor (gper) in scales of sea bass and tilapia, determined by RT-PCR and using as internal control the 18S ribosomal RNA

- In sea bass, DBP at 10^{-8}M induced a rapid and significant increase in TRAP activity. OP induced both rapid and slow (24h) increases in TRAP activities, depending on the dose
- No significant changes in ALP activity were observed for both compounds
- In tilapia, DBP decreased TRAP activity after 24h at the higher dose, while 10^{-8} M OP decreased ALP activity after 24h
- The expression of nuclear estrogen receptors (mainly of the ERβ subtype) and of a membrane estrogen receptor (GPER, at low levels) was detected in the scales of both species
- The results suggest that both compounds could cause an increase in calcium mobilization (indicated by TRAP activity) in the marine species sea bass, which resemble the well described effect of E2
- The decrease in TRAP and ALP activities in the fresh water tilapia suggests a tendency to slow down normal calcium turnover
- The detected estrogen receptors could mediate the observed disruptive actions in fish scales, including both rapid and long term responses, probably via GPER and nuclear ERs, respectively

CONCLUSIONS

- Exposure to DBP and OP may have disruptive effects on the metabolism of mineralized tissues in both marine and freshwater species
- Their mechanisms of action remain unknown but may involve disruption of the estrogenic system, since these pollutants have previously been found to bind fish estrogen receptors and disrupt other estrogenic regulated processes in fish
- Both compounds may act through both rapid and slow responses, potentially via different estrogen receptors
- Future studies will investigate the mechanisms involved in these responses and the consequences for fish health

References: Agas, D. *et al.* Arch. Toxicol. 2013, 87, 735–751; Kloas, W. *et al.* Gen. Comp. Endocrinol. 2000, 119, 287–299; Pinto, P.I. *et al.* Gen. Comp. Endocrinol. 2009, 160, 19–29; Pinto, P.I. *et al.* Mar. Drugs 2014, 12, 4474-4494; Rider, C.V. *et al.* Env. Toxicol. Chem. *2009,* 28, 2175–2181; Rotllant, J. *et al.* Regul. Pept. 2005, 132, 33–40; Suzuki, N. & Hattori, A. Life Sci. 2003, 73, 2237–2247.

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