Triphenyltin alters lipid homeostasis in females of the ramshorn snail Marisa cornuarietis

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Short-term exposure to the fungicide TPT disrupts lipid metabolism in M. cornuarietis at environmentally realistic concentrations.

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ABSTRACT

Molluscs are sensitive species to the toxic effects of organotin compounds, particularly to masculinisation. Both tributyltin (TBT) and triphenyltin (TPT) have been recently shown to bind to mollusc retinoid X receptor (RXR). If RXR is involved in lipid homeostasis, exposure to TPT would have an immediate effect on endogenous lipids. To test this hypothesis, the ramshorn snail Marisa cornuarietis was exposed to environmentally relevant concentrations of TPT (30, 125, 500 ng/L as Sn) in a semi-static water regime for 7 days. Percentage of lipids and total fatty acid content decreased significantly in TPT-exposed females while the activity of peroxisomal acyl-CoA oxidase, involved in fatty acid catabolism, increased. In addition, fatty acid profiles (carbon chain length and unsaturation degree) were significantly altered in exposed females but not in males. This work highlights the ability of TPT to disrupt lipid metabolism in M. cornuarietis at environmentally realistic concentrations and the higher susceptibility of females in comparison to males.

1. Introduction

Since the late 1960s, organotin compounds, such as tributyltin (TBT) and triphenyltin (TPT), have been extensively used across the world as biocides in antifouling paints, applied on ship hulls and fishing nets, and as fungicides in agricultural crops. Despite their gradual removal from the market and their prohibition of use, their release into the environment combined with their low solubility in water and high octanol–water partition coefficient has resulted in worldwide contamination of the aquatic environment (Fent, 1996). Both TBT and TPT are potent endocrine disruptors; abnormalities in the endocrine system related to TBT and TPT exposure have been observed in vertebrates (Iguchi et al., 2007; Kanayama et al., 2005; McAllister and Kime, 2003) and invertebrates (Alzieu, 2000; Oehlmann et al., 2007), with gastropods and oysters being among the most susceptible organisms. A concentration of 1 ng/L TBT is enough for the induction of imposex (superimposition of male secondary sexual characteristics, including a penis and vas deferens) in females of the gastropod Nucella lapillus (Bryan et al., 1986; Spooner et al., 1991).

Imposex has been reported in over 150 species of gastropods worldwide (Horiguchi et al., 1997), including Marisa cornuarietis following exposure to TBT and TPT (Schulte-Oehlmann et al., 1995, 2000). Although the link between imposex in female gastropods and exposure to TBT or TPT has been established, the exact mechanism through which this phenomenon occurs remains unclear. Imposex induction has been related to increased levels of testosterone and an inhibition of aromatase activity (Bettin et al., 1996), inhibition of the esterification of testosterone resulting in high levels of free testosterone (LeBlanc et al., 2005) or alterations in the excretion of neurohormones that contribute to sexual differentiation in gastropods (Oberdörster and McClellan-Green, 2002), among others.

Recent studies indicate that TBT and TPT may act through interaction with nuclear receptors (Nakanishi, 2007). Both TBT and TPT bind to the human Retinoid X receptor (hRXR) with high affinity, similar to that of 9-cis retinoic acid (9-cis RA), a proposed natural ligand of RXR (Nishikawa et al., 2004). RXR homologues have been cloned from the gastropods Thais clavigera (Nishikawa et al., 2004) and N. lapillus (Castro et al., 2007), as well as from the freshwater snail Biomphalaria glabrata (Bouton et al., 2005). All three show high similarity with vertebrate RXR and 9-cis RA was...
a high affinity ligand, which suggests that retinoid signaling pathways may exist in these species. Moreover, injections of T. clavigera and N. lapillus with 1 μg/g 9-cis RA induced imposex, leading to an increase in penis length and vas deferens similar to that induced by TBT and/or TPT in these species (Castro et al., 2007; Nishikawa et al., 2004).

In mammals, RXR forms heterodimers with orphan nuclear receptors (whose endogenous ligand is unknown: peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), farnesoid X receptor (FXR), and pregnane X receptor (PXR)) as well as with the retinoic acid, thyroid hormone and vitamin D receptors (Szanto et al., 2004). These orphan receptors are lipid sensors as they get activated by lipid molecules and therefore play an important role in lipid homeostasis, whereas the later regulate the endocrine system and resemble more closely the action of steroid hormone receptors (Chawla et al., 2001). Those RXR heterodimers are activated by ligands of either receptors and subsequently bind to the corresponding response elements in the promoter region of the target genes to modulate their transcription (Michalik et al., 2006). Knocking out RXR in mice disturbed lipid metabolism functions controlled by PPARα, PPARγ, LXRα, FXR and PXR (Szanto et al., 2004) showing the importance of this receptor in lipid homeostasis. Interestingly, TBT and TPT activate both RXR and PPARγ human receptors (Kanayama et al., 2005) and exposure of mice and the amphibian Xenopus laevis to TBT and RXR/PPARγ ligands stimulated lipid accumulation and ectopic adipocyte formation, respectively (Grin et al., 2006).

Additionally, activation of RXR and/or PPARs has been linked to alterations in the steroidogenic pathway: modulation of STAR expression, coupled to the oxidation of leuco-DCF in a reaction catalysed by exogenous peroxidase. The method was modified after Small et al. (1985). The digestive gland/gonad complex (0.3–0.7 g) from each M. cornuarietis individual was homogenized in Tissue buffer pH 7.6 (4 ml buffer/g of tissue), containing 1 mM sodium bicarbonate, 0.1 M EDTA, 0.1% ethanol and 0.01% Triton X-100. After homogenisation, samples were centrifuged at 500 × g for 15 min and the supernatant containing the peroxisomes was assayed for acyl-CoA oxidase activity. The reaction was carried out at 25°C in a final volume of 1 ml. The reaction mixture contained 0.05 mM leuco-DCF (prepared weekly by hydrolysing 2.66 mM H2DCF diacetate in 1.9 V/cm, dimethylformamide:NaOH (0.01 M) and stored at −20°C). 0.07 mg horseradish peroxidase, 40 mM sodium azide, 0.01% Triton X-100, 10 mM potassium phosphate buffer pH 7.4 and sample. This mixture was pre-incubated in the dark for 3 min, as some impurities in the peroxidase cause a small amount of oxidation of the leuco-DCF/PPARγ (Kochi and von Wartburg, 1978). After this time, the slow rate of auto-oxidation of the dye was determined by measuring spectrophotometrically the absorbance at λ = 502 nm for 2 min. The reaction was then started by the addition of 30 μM palmitoyl-CoA, and after 15 s of incubation in the dark, the enzymatic reaction rate was determined for 2 min. Rates were corrected by subtracting the blank (the addition of palmitoyl-CoA) and calculations were made by using a DCF molar extinction coefficient of 91,000 M−1 cm−1 as obtained by Kochi and von Wartburg (1978) from the peroxidase-catalysed oxidation of leuco-DCF. Protein concentrations were determined by the method of Lowry et al. (1951) by using bovine serum albumin as a standard.

2.2. Animals

Ramshorn snails, M. cornuarietis (Mollusca: Prosobranchia: Ampullariidae), came from a laboratory breeding stock which was derived from a stock at Aquazoo Düsseldorf (Germany) in 1991 with regular cross-breeding of wild-caught animals from Florida (USA) to avoid inbreeding. The breeding stock was kept in a flow-through system with fully reconstituted water under constant conditions regarding temperature and light dark cycle (12:12 h). Water parameters (pH, temperature, conductivity, nitrite, oxygen concentration and saturation) were measured twice a week per tank. Parameters of the fully reconstituted influent water were pH 7.5, 850 μS/cm, <1 mg NO2/L and >95% O2 saturation.

2.3. Exposure experiment

For the exposure experiments, two replicate groups of 17 sexually mature snails each were exposed to three nominal concentrations of TPT (30, 125, and 500 ng as Sn/l) for 7 days (June 2005) in fully reconstituted water at 24 ± 1°C. TPT was added in absolute ethanol, the concentration of ethanol in water being 0.001% in all experimental groups, including solvent control (SC). Test concentrations were selected based on results from earlier studies in M. cornuarietis (Jener et al., 2006) and on reported values of TPT in the aquatic environment (Becker-van Slooten and Tarradellas, 1995). The exposure experiment was performed in 40 l glass aquaria fitted with an Eheim filter system and additional aeration under 12-h light/12-h dark cycles. The exposure system was designed as semi-static renewal with addition of the test substance every 24 h (weekend 48 h) and 50% exchange of the water twice a week. Water parameters (pH, conductivity, temperature, nitrite, O2 concentration and saturation) were measured twice a week before the water was changed. Animals were fed daily with TetraMin® (Tetra, Melle, Germany) ad libitum. Exposed organisms were cooled in ice, the digestive gland/gonad complex was dissected, deep-frozen in liquid nitrogen, and stored at −80°C for determination of steroid levels and enzymatic activities.

2.4. Peroxisomal fatty acyl-CoA oxidase activity

Acyl-CoA (palmitoyl-CoA) oxidase was assayed by the determination of H2O2 production, coupled to the oxidation of leuco-DCF in a reaction catalysed by exogenous peroxidase. The method was modified after Small et al. (1985). The digestive gland/gonad complex (0.3–0.7 g) from each M. cornuarietis individual was homogenized in TVE buffer pH 7.6 (4 ml buffer/g of tissue), containing 1 mM sodium bicarbonate, 0.1 M EDTA, 0.1% ethanol and 0.01% Triton X-100. After homogenisation, samples were centrifuged at 500 × g for 15 min and the supernatant containing the peroxisomes was assayed for acyl-CoA oxidase activity. The reaction was carried out at 25°C in a final volume of 1 ml. The reaction mixture contained 0.05 mM leuco-DCF (prepared weekly by hydrolysing 2.66 mM H2DCF diacetate in 1.9 V/cm, dimethylformamide:NaOH (0.01 M) and stored at −20°C). 0.07 mg horseradish peroxidase, 40 mM sodium azide, 0.01% Triton X-100, 10 mM potassium phosphate buffer pH 7.4 and sample. This mixture was pre-incubated in the dark for 3 min, as some impurities in the peroxidase cause a small amount of oxidation of the leuco-DCF/PPARγ (Kochi and von Wartburg, 1978). After this time, the slow rate of auto-oxidation of the dye was determined by measuring spectrophotometrically the absorption at λ = 502 nm for 2 min. The reaction was then started by the addition of 30 μM palmitoyl-CoA, and after 15 s of incubation in the dark, the enzymatic reaction rate was determined for 2 min. Rates were corrected by subtracting the blank (the addition of palmitoyl-CoA) and calculations were made by using a DCF molar extinction coefficient of 91,000 M−1 cm−1 as obtained by Kochi and von Wartburg (1978) from the peroxidase-catalysed oxidation of leuco-DCF. Protein concentrations were determined by the method of Lowry et al. (1951) by using bovine serum albumin as a standard.

2.5. Fatty acid analysis

The digestive gland/gonad complex of M. cornuarietis individuals (0.3–0.7 g) was lyophilised and processed for lipid and fatty acid analysis. Lipids were extracted from the lyophilised samples by homogenisation in 2 ml ice-cold chloroform/methanol (2:1 v/v) plus 0.01% (v/v) butylated hydroxytoluene (BHT) as an antioxidant, following a modification of the method of Folch et al. (1957). After homogenisation, 0.25 ml of 0.88% KCl was added to the homogenate and the solution was mixed. After phase separation, the chloroform layer was removed, filtered and the solvent evaporated by flushing with nitrogen. The solid residue was then weighed to determine the total lipid levels, and afterwards redissolved in chloroform/methanol (2:1 v/v) with 0.01% BHT, flushed with nitrogen and stored at −20°C in a screw cap vessel. Lipid aliquots were transmethyllated overnight (Christie, 1982) after addition of a known amount of nonadecanoic acid (19:0) as internal standard (Sigma). Fatty acid methyl esters (FAME) were extracted with hexane/diethyl ether (1:1, v/v), and purified by thin layer chromatography (silica gel G60, Merck) using hexane/diethyl ether/acetic acid (85:15:1.5, v/v/v) as solvent system. FAME were analysed with a Fisons 8000 gas chromatograph equipped with a fused silica 30 × 0.25 mm open tubular column (Tracer, TR-WAX, film thickness: 0.25 μm), and a cold on-column injection system, using helium as carrier, and a 50–220°C thermal
graduates. Peaks were recorded and integrated in a personal computer using Azur software (Datalys, France), and identified by comparison with a well characterized sardine oil named Marinol (Fishing Industry Research Institute, Rosebank South Africa).

2.6. Statistical analysis

Results are mean values ± SEM. Differences between control groups (control and solvent control) were assessed with Student’s t-test and they were not statistically significant (p < 0.05). Thereafter, exposure groups were compared with the average of the control and solvent control groups by using one way ANOVA with Dunnett’s post hoc test.

3. Results

3.1. Peroxisomal acyl-CoA oxidase activity

AOX activity was determined in peroxisomal enriched fractions obtained from the digestive gland/gonad complex of M. cornuarietis individuals. After one week exposure to TPT, the activity AOX was significantly increased in females exposed to 30 and 500 ng TPT-Sn/L, resulting in 1.3- and 1.4-fold increase, respectively (Fig. 1). AOX activity was also significantly increased (1.4-fold) in males exposed to 30 ng TPT-Sn/L, but no further differences were observed at higher TPT concentrations (Fig. 1).

3.2. Fatty acid profile and lipid content

A detailed description of the fatty acid composition of control and exposed males and females of M. cornuarietis is given in Table 1. At least 33 fatty acids with carbon atoms from 14 to 24 were detected in the digestive gland/gonad complex of both males and females. Unsaturated fatty acids were the major group, accounting for 54% and 61% of total fatty acids in control males and females, respectively. Within this group, mono-unsaturated fatty acids (MUFA) constituted 28–30% of the total fatty acids and poly-unsaturated fatty acids (PUFA) 26–31%. The most abundant unsaturated fatty acids were linoleic (18:2n-6) and oleic (18:1n-9) acids. Highly unsaturated fatty acids (3 or more saturations) of 20 or more atoms of carbon (HUFA) represented only about 10% of total fatty acids. The major forms were in decreasing order, arachidonic (20:4n-6), docosahexaenoic (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3). Saturated fatty acids represented 23% of total fatty acids in males and 30% in females, and among them palmitic (16:0) and stearic (18:0) were the most abundant.

One week exposure to TPT caused a shift in the fatty acid profile in the digestive gland/gonad complex of M. cornuarietis with alterations being more evident in females (Table 1). Thus, a ~ 10% decrease of MUFA (% FAME) and a ~20% increase of HUFA were observed in females exposed to 125 and 500 ng TPT-Sn/L; the increase in HUFA was associated to a relative increase of the n-6 HUFA group. To further understand the effect of TPT exposure on individual fatty acids, those were expressed as mg/g dry tissue. Interestingly, one week exposure to TPT resulted in a decrease in fatty acids, both in terms of chain length and saturation degree, and this decrease was mainly detected in females (Tables 2 and 3). Almost all exposed females revealed a decrease in fatty acid content in terms of chain length (Table 2). C18 fatty acids that accounted for 50% of total fatty acids in the control groups showed a 40% decrease in females exposed to 30 and 500 ng TPT-Sn/L, whereas no significant alteration was observed in exposed males. In terms of unsaturation degree, saturated (ΣC:0), mono-unsaturated (ΣC:1) and di-unsaturated (ΣC:2) fatty acids, which account for 32, 34 and 23% of total fatty acids in the control groups, decreased in TPT-exposed females (30–40%) (Table 3). Only the tetra-unsaturated fatty acids (ΣC:4) were not significantly altered by TPT exposure in females; within this group, arachidonic acid (20:4n-6) was the most abundant (97%).

Overall, one week exposure to 30, 125 and 500 ng TPT-Sn/L resulted in a drop in the total fatty acid levels (FAME) in females equal to 33, 20 and 35% (Fig. 2). TPT caused a significant decrease (20%) in total fatty acid levels in males as well but only at the highest TPT concentration (Fig. 2). Furthermore, TPT exposure resulted in a significant decrease (20%) in the percentage of lipids in the digestive gland/gonad complex of females at the highest TPT concentration (500 ng/L as Sn) but had no significant effect in exposed males (Fig. 2).

4. Discussion

One week exposure to TPT had a significant effect on the percentage of lipids, fatty acid content and fatty acid metabolism in the digestive gland/gonad complex of females of M. cornuarietis, whereas males demonstrated very few significant alterations. Percentage of lipids, total fatty acid content as well as carbon chain length and unsaturation degree, all decreased significantly in TPT-exposed females. In parallel, the activity of peroxisomal AOX, the enzyme responsible for the breakdown of C14–C18 and C > 20 fatty acids was significantly induced; this supports the observed decrease in fatty acid content.

Activation of the enzymes involved in the peroxisomal β-oxidation pathway, including AOX, with a parallel increase in volume and density of peroxisomes is a phenomenon known as peroxisome proliferation that has been related to hepatocarcinogenesis in rats and mice (Yu et al., 2003). In vertebrates, the peroxisomal AOX gene is transcriptionally activated by PPARs (Reddy and Hashimoto, 2001). Although PPARs have not been identified in invertebrates (Thornton, 2003), existing data
demonstrate that peroxisome proliferation in response to inorganic contaminant exposure occurs. Thus, induction of AOX activity with a parallel increase in peroxisomal volume density has been observed in mussels *Mytilus edulis* exposed to specific peroxisome proliferators (fibrates and phthalates) and various organic pollutants (PAHs and PCBs) (Cajaraville and Ortiz-Zarragoitia, 2006; Ortiz-Zarragoitia and Cajaraville, 2006), in slugs *Arion ater* exposed to a Cd-kerosene mixture (Zaldibar et al., 2007) and in the land snail *Helix aspersa* exposed to airborne urban pollutants (Regoli et al., 2006). This indicates that a mechanism of peroxisome proliferation analogous to the one promoted by PPARs activation in vertebrates may also exist in invertebrates.

The fact that total lipid content and almost all fatty acid groups decreased in such a short exposure period in exposed females is of special concern, taking into account the multi-functional role of fatty acids in cell structure and function, energy metabolism and storage, bioactive signaling and synthesis of various compounds involved in physiological regulation (e.g. steroids, eicosanoids, etc.) (Benati et al., 2004). Toxicity of organochlorines has been related to their interference with membrane permeability, fluidity and signaling (Ortiz et al., 2005). Thus, exposure of *Ostrea edulis* to TBT for 23 h caused a reduction in total lipids and

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### Table 1

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Males</th>
<th>SC</th>
<th>C</th>
<th>30 mg/L</th>
<th>125 mg/L</th>
<th>500 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:0</td>
<td>1.57</td>
<td>1.55</td>
<td>1.58</td>
<td>1.60</td>
<td>1.62</td>
<td>1.65</td>
</tr>
<tr>
<td>20:4n-3</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
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</tbody>
</table>

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### Table 2

<table>
<thead>
<tr>
<th>TPT</th>
<th>SC</th>
<th>C</th>
<th>30 mg/L</th>
<th>125 mg/L</th>
<th>500 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14</td>
<td>0.62</td>
<td>0.04</td>
<td>0.70</td>
<td>0.10</td>
<td>0.70</td>
</tr>
<tr>
<td>C15</td>
<td>0.22</td>
<td>0.01</td>
<td>0.65</td>
<td>0.08</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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Values are expressed as % of total fatty acid methyl esters (mean ± SEM; n = 4). Significant differences respect to controls indicated by *p < 0.05* and **p < 0.01**.
triglycerides but an increase in phospholipids and PUFA, including HUFA and arachidonic acid (Puccia et al., 2005); phospholipids and PUFA are involved in maintaining membrane fluidity and the authors suggest that this increase is an adaptive mechanism to TBT toxicity. In the present work, a relative increase of arachidonic acid was observed in 7 days exposed females (Table 1). Arachidonic acid is involved in cell signaling and specifically as a substrate for eicosanoids synthesis (Nakamura and Nara, 2004). Thus, the relative increase of arachidonic acid (30%) detected in the present work may be a short-term response in exposed females that will allow them to maintain endogenous levels of arachidonic stable (2.0–2.4 mg/g) and therefore minimize the effect of TPT on physiological functions.

Additionally, the proportion of arachidonic acid was markedly increased in females exposed to 125 and 500 ng/L TPT-Sn, but not in males (Table 1). These changes paralleled the alterations observed in total lipid and fatty acid levels, suggesting a link between the relative increase of this potential regulator of lipogenesis (Yoshikawa et al., 2002) and the observed decrease in total lipid and fatty acid levels in exposed individuals.

Fatty acids are endogenous ligands of various nuclear steroid receptors in vertebrates. Palmitic (16:0), stearic (18:0), palmitoleic (16:1n-7), oleic (18:1n-9), linoleic (18:2n-6), arachidonic acid (20:4n-6) and EPA (20:5n-3) are endogenous ligands of PPARs, which is involved in fatty acid oxidation and catabolism; whereas linoleic, arachidonic acid and eicosanoids are endogenous ligands of PPARγ that plays a central role in adipocyte differentiation and storage of fatty acids (Willson and Wahl, 1997; Reddy and Hashimoto, 2001; Kota et al., 2005; Mochizuki et al., 2006). Other transcriptional factors have been identified to be targets of fatty acid regulation such as the Liver X receptor and RXR, which are both involved in lipid regulation (Benatti et al., 2004). Since fatty acids appear to be PPAR ligands at a concentration range that is consistent with their physiological circulating levels (Braissant et al., 1996), alterations in the abundance of endogenous fatty acids may trigger different mechanisms of lipid regulation further down the cascade of events. Indeed, Janer et al. (2007) found a significant increase in the percentage of total lipids and total fatty acid content in females of M. cornuarietis exposed to 500 ng TBT-Sn/L for 100 days. Furthermore, the percentage of PUFAs, including HUFA, decreased and MUFAs increased. The discrepancies with the present study are probably a reflection of long- and short-term effects of organotin compounds on lipid homeostasis rather than a different effect of TBT and TPT, and deserve further investigation. Interestingly, both studies indicated higher susceptibility of females than males of M. cornuarietis to lipid alterations.

Additionally, in some invertebrates, steroids are conjugated with fatty acids to form apolar esters. These esters are retained in the lipoidal matrices of the body from where they can be hydrolysed by esterases and liberate the active steroids upon demand (Borg et al., 1995). Most of the endogenous estradiol and testosterone detected in the digestive gland/gonad complex of M. cornuarietis are in the esterified form (Janer et al., 2006). Esterification of steroids occurs upon acyl-CoA moieties, whose activation is dependent on the concentration of the corresponding fatty acids (Hochberg, 1998). Estradiol esters formation was achieved using the fatty acid moieties C16:0, C16:1, C18:0, C18:1, C18:2 and C20:4 in the oyster Crassostrea virginica (Janer et al., 2004). Exposure of mussels M. edulis to estradiol resulted in the formation of estradiol esters with C16:0, C16:1 and C16:2 fatty acid moieties (Labadie et al., 2007). Interestingly, in the present work, one week exposure to TPT resulted in a significant increase in esterified testosterone levels (60–85%) and a concomitant decrease in esterified estradiol (50–84%) (Lyssimachou et al., 2008). The observed alterations in esterified steroids were not directly related to changes in P450 aromatase activity or changes in 17β-hydroxysteroid dehydrogenase or 5α-reductase, two enzymes involved in the metabolism of the androgen precursor androstenedione in M. cornuarietis (Lyssimachou et al., 2008). Thus,

### Table 3

Levels of fatty acids grouped by unsaturation degree in the digestive gland/gonad complex of Marisa cornuarietis exposed to different concentrations of TPT (ng/L as Sn) for 7 days.

<table>
<thead>
<tr>
<th>TPT (ng as Sn/L)</th>
<th>C</th>
<th>SC</th>
<th>30 ng/L</th>
<th>125 ng/L</th>
<th>500 ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C:0</td>
<td>16.76 ± 0.68</td>
<td>16.60 ± 1.32</td>
<td>12.53 ± 2.51</td>
<td>13.69 ± 0.19</td>
<td>10.89 ± 0.63</td>
</tr>
<tr>
<td>C:1</td>
<td>17.72 ± 1.34</td>
<td>18.31 ± 1.97</td>
<td>11.12 ± 1.96</td>
<td>13.66 ± 0.33</td>
<td>11.10 ± 0.40</td>
</tr>
<tr>
<td>C:2</td>
<td>10.69 ± 0.58</td>
<td>13.56 ± 1.43</td>
<td>7.25 ± 1.12</td>
<td>10.26 ± 0.28</td>
<td>8.29 ± 0.41</td>
</tr>
<tr>
<td>C:3</td>
<td>0.79 ± 0.04</td>
<td>1.06 ± 0.12</td>
<td>0.55 ± 0.07</td>
<td>0.77 ± 0.04</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>C:4</td>
<td>2.06 ± 0.09</td>
<td>2.56 ± 0.25</td>
<td>2.19 ± 0.23</td>
<td>2.53 ± 0.20</td>
<td>2.05 ± 0.22</td>
</tr>
<tr>
<td>C:5</td>
<td>1.25 ± 0.13</td>
<td>1.57 ± 0.12</td>
<td>1.08 ± 0.09</td>
<td>1.25 ± 0.04</td>
<td>1.12 ± 0.07</td>
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<tr>
<td>C:6</td>
<td>1.22 ± 0.16</td>
<td>1.50 ± 0.17</td>
<td>0.75 ± 0.13</td>
<td>1.11 ± 0.03</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C:0</td>
<td>19.42 ± 2.14</td>
<td>17.78 ± 0.90</td>
<td>20.39 ± 1.42</td>
<td>22.14 ± 1.53</td>
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<tr>
<td>C:1</td>
<td>16.83 ± 2.83</td>
<td>14.56 ± 1.89</td>
<td>17.38 ± 2.06</td>
<td>19.42 ± 1.87</td>
<td>13.18 ± 2.23</td>
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<tr>
<td>C:2</td>
<td>9.49 ± 2.30</td>
<td>8.19 ± 1.15</td>
<td>10.48 ± 1.73</td>
<td>9.96 ± 2.06</td>
<td>5.65 ± 1.28</td>
</tr>
<tr>
<td>C:3</td>
<td>0.68 ± 0.22</td>
<td>0.76 ± 0.08</td>
<td>1.03 ± 0.14</td>
<td>0.94 ± 0.17</td>
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<td>C:4</td>
<td>2.18 ± 0.27</td>
<td>1.90 ± 0.29</td>
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<td>1.97 ± 0.20</td>
<td>1.52 ± 0.13</td>
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<td>C:5</td>
<td>1.79 ± 0.34</td>
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<td>1.93 ± 0.25</td>
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<td>1.05 ± 0.29</td>
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<tr>
<td>C:6</td>
<td>1.84 ± 0.54</td>
<td>1.38 ± 0.30</td>
<td>2.04 ± 0.37</td>
<td>1.60 ± 0.34</td>
<td>0.80 ± 0.19</td>
</tr>
</tbody>
</table>

Values are expressed as mg/g of dry weight (mean ± SEM; n = 4). Significant differences respect to controls indicated by *p < 0.05 and **p < 0.01.
the hypothesis that changes in fatty acid availability might trigger alterations in endogenous steroid levels is a challenging one. In molluscs, the esterification of steroids with fatty acids appears to be an important regulation mechanism of endogenous steroid levels (Gooding and LeBlanc, 2001; Janer et al., 2005).

Overall, short-term exposure of M. cornuarietis to environmentally relevant doses of TPT leads to a decrease of total lipids and fatty acid content and an increase in AOX activity, which is involved in fatty acid catabolism. Since fatty acids have a pivotal role in organisms (cell membrane composition, bioactive signaling, steroid and eicosanoid synthesis), the observed effects are of special concern. Further research should focus on the higher sensitivity of females in comparison to males, the potential link of these alterations in endogenous steroid levels is a challenging one. In particular, the regulation of endogenous steroid levels is important as a result of exposure to estrogens (Hochberg, 1998).

Finally, being TPT a high affinity ligand of RXR, the obtained data further support the hypothesis that RXR may also be implicated in lipid homeostasis in gastropods (alone or in combination with putative PPARs) as it is in vertebrates.

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References


