TESTOSTERONE AND ENERGY METABOLISM IN THE ESTUARINE MYSID *NEOMYSIS INTEGER* (CRUSTACEA: MYSIDACEA) FOLLOWING EXPOSURE TO ENDOCRINE DISRUPTORS

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Abstract—A diverse set of reference compounds suspected of having an endocrine-disrupting mode of action (i.e., testosterone, flutamide, ethinylestradiol, precocene, nonylphenol, fenoxycarb, and methoprene) were tested for acute toxicity to the estuarine mysid Neomysis integer (Crustacea: Mysidacea). Neomysis integer was very sensitive to all tested compounds, with 96-h median lethal concentrations in a narrow range between 0.32 and 1.95 mg/L. The pesticides methoprene and fenoxycarb, both synthetic insect juvenile hormone analogs, were most toxic to *N. integer*. In addition, the short-term sublethal effects of methoprene and nonylphenol (an estrogen agonist) on the energy and steroid metabolism of *N. integer* were evaluated. Both compounds significantly affected energy and testosterone metabolism of *N. integer* at concentrations below acute toxicity levels. Energy consumption in methoprene- and nonylphenol-exposed mysids was significantly induced at 100 µg/L, resulting in a lower cellular energy allocation in these animals. Testosterone phase I metabolism was affected at 10 µg/L, whereas glycosylation was the most important phase II pathway affected in mysids exposed to 100 µg/L of both compounds. Methoprene exposure resulted in a concentration-dependent increase in the metabolic androgenization ratio. Mysids exposed to nonylphenol at 10 µg/L had a significantly higher metabolic androgenization ratio. The present study indicates that energy and testosterone metabolism of mysids, as endpoints, are able to detect endocrine-disruptive activity of chemicals after short-term exposure to environmentally realistic levels of endocrine disruptors.

Keywords—Neomysis integer  Testosterone metabolism  Energy metabolism  Endocrine disruption  Biomarker

INTRODUCTION

Invertebrates account for approximately 95% of all known species of animals on our planet, yet our knowledge of their basic endocrinology is limited, and only recently have we begun to understand their value in signaling environmental endocrine disruption [1]. The majority of evidence for chemically induced endocrine disruption in invertebrates stems from exposures to pesticides specifically designed to disrupt endocrine-regulated processes such as growth, metamorphosis, and molting [2]. Historically, most assays used to evaluate endocrine function in invertebrates have involved the use of general endpoints that did not reveal specific mechanisms. Fortunately, new and more sophisticated analytical techniques allow analyses of specific pathways and use microquantities of biological material [3]. Consequently, a number of new hormone-regulated endpoints have been proposed as promising biomarkers to detect endocrine disruption in invertebrates [1]. We presently are investigating the use of mysid shrimp as an invertebrate test model for the evaluation of environmental endocrine disruption.

Chronic toxicity tests with invertebrates such as daphnids (*Daphnia* and *Ceriodaphnia*) and mysid shrimp (*Americanmysis*) have been required for decades for the evaluation of the toxicity of environmental pollutants. The short life cycle of these organisms allows for the measurement of endocrine-regulated endpoints in a time frame sufficiently short to qualify as screening tests [1,3]. The endpoints currently used in acute and chronic toxicity testing are intended to define organism or population status (e.g., survival and growth). These endpoints may not be the most suitable endpoints for detecting chemicals that have an endocrine-disrupting mode of action. Presently, the most appropriate evaluation criteria cannot be identified because data to select the most discriminating endpoint are lacking. In the absence of data that can suggest that existing tests and effect assessment procedures adequately protect invertebrate communities, the number of new endpoints or new test species might increase [3].

Mysid shrimp may serve as viable surrogates for many crustaceans and have been put forward as suitable test organisms for the evaluation of endocrine disruption [1,3]. In addition, we recently demonstrated that alterations in energy and steroid metabolism are useful biomarkers to evaluate the effects of chemicals on mysids [4,5]. To validate the use of these assays as sensitive endpoints to detect the potential effects of chemicals with endocrine-disrupting properties in mysids, we presently are evaluating relative endpoint responses via exposure experiments with a selection of reference chemicals. A list of reference chemicals (based on their possible mode of action) for evaluating endpoint or species sensitivity to potential endocrine-disrupting compounds was suggested at a Society of Environmental Toxicology and Chemistry workshop on endocrine disruption in invertebrates held in The Netherlands in 1998 [1]. This list includes methoprene (juvenile hormone agonist), precocene (juvenile hormone antagonist), 20-OH ecdysone (ecdysone agonist), homobrassinolide and...
luteolin (ecdysonic antagonists), fadrozole (aromatase inhibitor), methyl testosterone (androgen agonist), flutamide (androgen antagonist), 4-tert-pentylphenol (weak estrogen agonist), ethinylestradiol (strong estrogen agonist), and ZM-189,154 (estrogen antagonist).

The present study investigates the acute toxicity of a number of these reference chemicals (i.e., testosterone, flutamide, ethinylestradiol, methoprene, and precocene) to the mysid Neomysis integer. In addition, another juvenile hormone agonist was included, the pesticide fenoxycarb, which has been reported to affect energy metabolism of juvenile mud crabs [6]. As an estrogen agonist, nonylphenol instead of 4-tert-pentylphenol was used, because this chemical has been demonstrated to alter testosterone metabolism in Daphnia magna [7]. Furthermore, N. integer is exposed to high concentrations of this chemical in the Scheldt estuary (The Netherlands) [8]. In addition to mortality, the effects of nonylphenol and methoprene on testosterone and energy metabolism of N. integer were evaluated after short-term exposures. Methoprene has been reported to significantly affect energy metabolism in the mysid shrimp Americanus bahia [9] and the development of secondary sex characteristics in daphnids has been reported to be altered by this compound [10]. Consequently, nonylphenol and methoprene are suitable reference compounds to evaluate steroid and energy metabolic processes in N. integer as biomarkers for endocrine disruption.

**MATERIALS AND METHODS**

**Chemicals**

Testosterone, methyltestosterone, flutamide, 17α-ethinylestradiol, precocene, nonylphenol, fenoxycarb, and methoprene were obtained from Sigma-Aldrich (Bornem, Belgium). Androstenedione, dihydrotestosterone, β-boldenone, and the different testosterone metabolites (2α-, 6α-, 6β-, 7α-, 11β-, 11β-, 15α-, 16α- and 16β-hydroxytestosterone) were purchased from Steraloids (Newport, RI, USA). Other solvents and reagents were analytical grade and purchased from Merck Eurolab (Leuven, Belgium).

**Animal collection and maintenance**

Initial populations of N. integer were collected from the shore by hand net in the Galgenweel (a shallow, brackish water near Antwerp, Belgium). After a short acclimatization period, the organisms were transferred to 200-L glass aquaria. The culture medium was artificial seawater (Instant Ocean®, Aquarium Systems, Sarrebourg, France) diluted with aerated, deionized tap water to a final salinity of 5‰. A 14:10-h light: dark photoperiod was used during culturing, and water temperature was maintained at 15°C. Cultures were fed daily with 24- to 48-h-old nauplii of Artemia ad libitum. Hatching of the cysts of Artemia was performed in 1-L, cylinder–conical vessels under vigorous aeration and continuous illumination at 25°C.

**Acute toxicity tests**

Juvenile mysids of equal size (visual selection of animals with a size of 2–4 mm) were taken from the laboratory culture and randomly distributed to 400-ml glass beakers containing 200 ml of the desired test concentration in water with a salinity of 5‰ (diluted from artificial seawater [Instant Ocean®] with deionized, carbon-filtered tap water). For each test concentration, two replicate beakers with five mysids were used. Mysids were exposed for 96 h to increasing concentrations of the test compounds testosterone, flutamide, ethinylestradiol, nonylphenol, methoprene, fenoxycarb, and precocene (Table 1). All compounds were delivered to the exposure solutions in absolute ethanol. The concentration of ethanol in the solvent control was 0.1%. Exposure temperature was 15 ± 1°C and exposure solutions were renewed after 48 h. Animals were fed twice daily with 24- to 48-h-old nauplii of Artemia (30–50 Artemia/mysid) and mortality was noted daily. At the end of the 96-h exposure period, the median lethal concentrations (LC50s) were calculated.

**Ninety-six-hour exposures to sublethal concentrations of nonylphenol and methoprene**

Juvenile mysids of equal size (average wet wt 2.9 ± 1.5 mg and 2.8 ± 1.4 mg in methoprene and nonylphenol experiments, respectively) were taken from the culture and randomly distributed to 400-ml glass beakers (five mysids/beaker) containing 200 ml of the desired test concentration in water with a salinity of 5‰. For each test concentration, 50 mysids were exposed for 96 h to sublethal concentrations of methoprene (control and 0.01, 1, or 100 μg/L) and nonylphenol (control and 0.01, 1, or 100 μg/L) and nonylphenol (control and 0.01, 1, or 100 μg/L) and nonylphenol (control and 0.01, 1, or 100 μg/L).
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Table 2. Median lethal concentrations (LC50s) for a number of endocrine disruptors to juvenile Neomysis integer. The 95% confidence interval is given in parentheses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>96-h LC50 (mg/L)</th>
<th>Literature LC50 (mg/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>1.95 (0.55–9.08)</td>
<td>5.6 (4.7–6.6)</td>
<td>Copepod [37]</td>
</tr>
<tr>
<td>Flutamide</td>
<td>1.38 (0.49–4.12)</td>
<td>5.4 (4.2–7.0)</td>
<td>Copepod [37]</td>
</tr>
<tr>
<td>17α-Ethinylestradiol</td>
<td>1.20 (0.39–3.78)</td>
<td>1.1 (NA*)</td>
<td>Copepod [37]</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>0.77 (0.24–2.26)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>0.59 (0.15–3.85)</td>
<td>0.1–0.5</td>
<td>Aquatic organisms [7]</td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>0.53 (0.16–1.51)</td>
<td>0.92 (NA)</td>
<td>Decapod [38]</td>
</tr>
<tr>
<td>Methoprene</td>
<td>0.32 (0.10–1.00)</td>
<td>0.125 (NA)</td>
<td>Mysid [9]</td>
</tr>
</tbody>
</table>

*NA = not available.

and 0.01, 1, or 100 μg/L). Further test conditions were identical to these of the acute toxicity tests.

At the end of the exposure period, 10 mysids were removed per concentration, and exposed for another 6 h to testosterone in 5-ml glass tubes containing 2 ml of the same toxicant test concentration to which 2 μg of testosterone (in 10 μl of methanol) was added. This additional exposure was used to evaluate the ability of mysids to eliminate testosterone as polar and nonpolar derivatives. The other mysids were directly shock-frozen in liquid nitrogen and kept at −80°C until analysis of the cellular energy allocation (CEA).

Testosterone and energy metabolism assays

The testosterone metabolism assay and the liquid chromatography with multiple mass spectrometry analyses were performed as described by Verslycke et al. [5] and Verslycke [8]. After the 6-h metabolic assay, in vivo–produced polar (hydroxylated) and nonpolar (reduced or dehydrogenated) phase I testosterone metabolites were extracted from the homogenized organisms by using 2 ml of ethyl acetate (1 ml two times) and phase-separated by centrifugation (5 min, 14,000 g). The two ethyl acetate fractions were pooled for analysis. Testosterone metabolites were extracted from the medium in the same way with 4 ml of ethyl acetate (2 ml two times). After ethyl acetate extraction, the remaining polar phase II testosterone metabolites in the medium were hydrolyzed. In short, the assay medium was evaporated and subsequently the testosterone metabolites in the medium were hydrolyzed.

The effects of 96 h of exposure to sublethal concentrations of methoprene (control and 0.01, 1, and 100 μg/L) on the testosterone and energy metabolism of N. integer are summarized in Figures 1 and 2.

Sublethal effects of methoprene on testosterone and energy metabolism of N. integer

The effects of 96 h of exposure to sublethal concentrations of methoprene (control and 0.01, 1, and 100 μg/L) on the testosterone and energy metabolism of N. integer are summarized in Figures 1 and 2. Methoprene had significant effects on the different pathways of energy allocation in N. integer. Of the individual energy reserve fractions, only proteins were significantly higher in the highest exposure concentration, compared with the control. The overall available energy (sum of protein, sugar, and lipid) was higher in methoprene-exposed mysids, although this was not statistically significant. However, energy consumption was significantly induced by methoprene exposure and was almost four times higher in the methoprene treatment at 100 μg/L compared with control mysids. This increased energy consumption resulted in significant alterations in the energy allocation of methoprene-exposed mysids, which had a lower CEA value, indicating that they were allocating more energy toward energy-demanding processes to cope with chemical exposure.
The effects on energy allocation also were reflected in the metabolic capacity of *Neomysis integer* to eliminate testosterone as polar and nonpolar derivatives. Phase I testosterone metabolism (hydroxylation and reductions and dehydrogenation) was significantly different in the lowest methoprene exposure concentration (10 ng/L) compared to control mysids. Elimination of testosterone as polar phase II conjugates was lower in methoprene-exposed mysids and this was significant for glycosylation in the highest exposure concentration. This reduction in the elimination of testosterone as polar metabolites results in a buildup of various nonpolar androgen-active metabolites such as androstenedione, dihydrotestosterone, and testosterone in the tissues of methoprene-exposed mysids. The overall impact of a chemical on the metabolic elimination of testosterone can be calculated by the metabolic androgenization ratio, which is the ratio of the rates of production of the eliminated reduced and dehydrogenated products and the polar products (hydroxylated plus conjugated). This ratio was significantly higher in the 100-mg/L treatment compared to the control.

**Sublethal effects of nonylphenol on testosterone and energy metabolism of *N. integer***

The effects of 96 h of exposure to sublethal concentrations (control and 0.01, 1, and 100 μg/L) of nonylphenol on the testosterone and energy metabolism of *N. integer* are summarized in Figures 3 and 4.

In general, the effects of nonylphenol on energy metabolism were similar to what was observed for methoprene-exposed mysids, that is, sugar content decreased, protein content increased, and lipid content was relatively unaffected. The overall effect of nonylphenol on the individual energy reserve fractions of *N. integer* resulted in a concentration-dependent increase in the total available energy. Similar to what was observed in the methoprene experiment, this increase in total available energy was not significantly different from the control treatment. In addition, a similar significant increase in energy consumption, as observed in methoprene-exposed mysids, was found in the highest nonylphenol concentration. This increased energy demand resulted in a decrease in the CEA in the highest exposure treatment; however, this decrease was not significant.

The effects of nonylphenol on testosterone metabolism were very different from what was observed for methoprene. Although a similar induction in oxidative metabolism was apparent in the lowest exposure concentration, the effects on phase I reductive metabolism and phase II conjugation were different. Glycosylation was significantly increased in the highest nonylphenol exposure concentration, whereas a sig-
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**Fig. 3.** Cellular energy allocation in *Neomysis integer* after 96 h of exposure to sublethal concentrations of nonylphenol (analysis of variance, Dunnett’s test; *p* < 0.05; **p** < 0.01; significantly different from control). Number below the whisker indicates the number of observations. *ww* = wet weight.

Significant reduction was observed in this treatment for methoprene. However, in both experiments, glycosylation appeared to be the major biochemical target of the tested compounds. Similar to what was observed with methoprene, phase II conjugation was strongly correlated with endogenous concentrations of vertebrate-type androgens, although the effects were opposite. The metabolic androgenization ratio was higher in all nonylphenol-exposed mysids, but this was only significant for the lowest nonylphenol exposure concentration (10 ng/L).

**DISCUSSION**

**Acute toxicity of the tested compounds to N. integer**

As mentioned in the introduction, the incentive for investigating whether the testosterone and energy metabolism are sensitive to endocrine-disrupting compounds was the finding that tributyltin (TBT) affects these processes at lower concentrations than those found to be acutely toxic [5]. Consequently, acute toxicity to N. integer was determined for a range of reference compounds, as shown in Table 2. From these experiments, the conclusion can be made that N. integer is at least as sensitive as and occasionally more sensitive to these compounds than other aquatic invertebrates previously tested. This corroborates earlier toxicity work with mysids, which demonstrated that they are among the most sensitive aquatic species to a wide range of chemicals [1,12–16].

**Sublethal effects of methoprene and nonylphenol on the energy metabolism of N. integer**

The effects of chemicals on the energy allocation of N. integer are consistent between the different exposure experiments. We previously investigated the effects of tributyltin chloride (TBTCl) and chlorpyrifos on CEA in this species after short-term exposure [5,8]. Both 48-h exposure to chlorpyrifos and 96-h exposure to TBTCl resulted in a significant increase in proteins, a decrease in sugars, and a relatively unaffected lipid fraction. The concentration-dependent increase in energy consumption results in a lower CEA, although the total available energy, in general, increases in toxicant-exposed mysids. This general scheme was confirmed with the experiments in this study, although the effects were stronger in methoprene-exposed mysids, compared to nonylphenol-exposed mysids. Olmstead and LeBlanc [17] found that daphnids exposed to methoprene at 50 and 100 μg/L for 3 and 6 d were significantly smaller. Mysids in our study exposed to methoprene at 100 μg/L had a significantly lower CEA, which indicates that less energy was available for growth and reproduction. This would corroborate the growth effects observed in daphnids. However,
the wet weight of methoprene-exposed mysids was not significantly different from the wet weight of control mysids, thus, not confirming this assumption after short-term exposure. Long-term exposures are needed to further elucidate potential effects of methoprene on mysid growth.

The 96-h LC50s indicate that methoprene is almost twice as toxic as nonylphenol, and this was also reflected in the CEA response, which was significantly reduced in the 100-μg/L treatment in methoprene-exposed mysids, but not in nonylphenol-exposed mysids. However, cellular respiration was significantly higher in the 100-μg/L treatment for both compounds, compared with control mysid respiration. As such, the no-observed-effect concentration for CEA in mysids after exposure to methoprene and nonylphenol was 1 and 100 μg/L, respectively. Previously, we found a 96-h lowest-observed-effect concentration (LOEC) for TBTCl of 10 ng/L (tested concentrations: control and TBTCl at 10, 100, and 1,000 ng/L) and a 48-h LOEC of 38 ng/L for chlorpyrifos (tested concentrations: control and chlorpyrifos at 38, 56, 72, and 100 ng/L). The respective LC50s for these compounds when using the same exposure duration was TBTCl at 164 ng/L [4] and chlorpyrifos at 270 ng/L [13]. The acute toxicity of these chemicals thus is clearly reflected in the different sensitivities in the CEA assays. In conclusion, the short-term sublethal effects of the chemicals tested in the present study on the CEA of N. integer are observed at concentrations that are 3 to 10 times lower than the LC50.

A previous study demonstrated that the biochemical endpoints of the CEA analysis are significantly related with population-level effects in daphnids, indicating the possibility of correlating energy-based suborganismal effects with those emerging at the higher levels of biological organization [18]. Furthermore, in these studies, the CEA criterion correlated much better with ecologically relevant population-level test criteria (i.e., mean brood size, mean number of young per female, net productive rate, and intrinsic rate of natural increase) than the individual energy fractions of the CEA, because of its integrated nature. The life history of mysids is very amendable to demographic modeling [19,20]. Therefore, the correlation of the individual biochemical endpoints, as well as the integrated CEA value, with population parameters in mysids should be an area of future research. However, the long life span of N. integer might limit the practical use of such assays. Clearly, the standard mysid test species A. bahia has the advantage over N. integer in allowing demographic modeling in much shorter periods [19,20]. Consequently, chronic pesticide exposure studies with A. bahia have demonstrated the ecological relevance and utility of short-term bioindicators of metabolic processes [21–24]. However, to date, the CEA has not been evaluated in chronic assays with mysids.

Sublethal effects of methoprene and nonylphenol on the testosterone metabolism of N. integer

The effects of methoprene and nonylphenol on the phase I oxidative elimination of testosterone were similar, and corroborate the results of a previous study with TBTCl [5] in which a comparable induction was observed in the lowest test concentration, but not at higher concentrations. Steroid hydroxylation reactions are catalyzed by P450 isozymes and, consequently, testosterone metabolism is a tool to study the activity of P450s in isozymes. Many xenobiotics, such as polycyclic aromatic hydrocarbons, can induce the expression of P450s [25,26]. Induced expression of P450s results in higher monooxygenase activity and the production of more polar derivatives of exogenous compounds, which are more easily excreted from the body. The induction of P450s only at low exposure concentrations could be due to several factors. First, the highest test concentrations may not have induced P450 enzymes. Second, the test compounds may block the activity of P450s at high concentrations, similar to what is observed for TBTCl [5,26]. Finally, phase II metabolism may be an important reducing factor in the formation of phase I hydroxylated metabolites [27]. We previously studied the inducibility of P450s in N. integer exposed to benzo[a]pyrene and found that 1 μg/L had a stronger effect than 50 μg/L, although P450 activities were very low at both exposure concentrations (Verslycke et al., unpublished data). Because we did not measure total P450 activity in the present experiment, it cannot be determined if the tested compounds induced these isozymes. Consequently, future studies are needed to evaluate P450 induction in mysids. We are not aware of studies that have demonstrated the binding and inactivation of P450 activity by nonylphenol or methoprene. However, it was remarkable that a strong correlation was observed between phase II glycosylation and phase I hydroxylation in the nonylphenol experiment, which might indicate that this chemical increases the activity of reductions and dehydrogenation and hydroxylation metabolic pathways resulting in the suppressed activity of conjugative pathways due to lack of substrate (testosterone). Baldwin et al. [7] observed a similar effect of nonylphenol on the elimination profile of testosterone in D. magna. However, they concluded that the unique response profile of the four metabolic processes (hydroxylation, reduction and dehydrogenation, glycosylation, and sulfation) suggests that nonylphenol directly and differentially altered each process. In our opinion, glycosylation probably was induced by nonylphenol exposure in mysids, resulting in less testosterone available for the phase I metabolism, and this was also reflected in a significant decrease in the endogenous concentrations of testosterone (Fig. 3). Similarly, the decrease in phase II glycosylation in mysids exposed to methoprene at 100 μg/L was correlated with an increase in endogenous androgen concentrations, which resulted in a significantly higher metabolic androgenization ratio.

At this time, it remains unclear how nonylphenol and methoprene affect the different processes of testosterone metabolism in N. integer. Although none of the above mechanisms can be excluded, we hypothesize that chemicals interact with the phase II metabolism of N. integer leading to the observed effects on metabolic buildup of potent androgens. This is consistent with the findings of studies by Ronis and Mason [28] and Oberdörster et al. [29], who evaluated testosterone metabolism in TBT-exposed invertebrates. Apparently, glycosylation may be the most important pathway in mysids, similar to what is observed in daphnids [7,30], although a previous study with TBTCl in N. integer found that sulfation was most strongly affected [5]. Future studies will have to determine which of these processes is most important in the detoxification of chemicals in mysids, or if these processes might be differentially affected depending on the chemical.

A final consideration is the observed metabolic androgenizing effect of methoprene observed in our study. Recently, Olmstead and Leblanc [10] demonstrated that methoprene can modulate specific components of the sexual reproductive phase of daphnids. Exposure to methoprene at 10 and 50 μg/L increased the percentage of male offspring produced in all broods in D. magna. The low concentrations at which methoprene
elicited these effects support the hypothesis that they are the consequence of this juvenile-hormone analog (juvenoid) acting at high-affinity receptors to endogenous juvenoids, rather than some overt toxicological response. Our results corroborate these findings because effects on steroid metabolism were apparent in the same concentration range (LOEC of 100 μg/L). Consequently, the developmental abnormalities observed in daphnids might be a result of methoprene altering testosterone metabolism, as was previously demonstrated for D. magna exposed to other compounds [3,31]. Mu and LeBlanc [32] demonstrated that embryotoxicity of testosterone in daphnids is due largely to its ability to interfere with ecdysoid control of development, probably through ecdysoid receptor antagonism. These observations suggest that steroid androgens have a specific target site of toxicity in daphnids and mysids. Although a functional role for steroidal androgens has not been firmly established, testosterone has been reported to have androgen-like activity in some crustaceans [3]. Furthermore, male daphnids have been demonstrated to be more susceptible to the toxicity of some chemicals than are females [17]. We have reported endogenous concentrations of testosterone and other vertebrate-type androgens in N. integer and have also observed a sex-specific steroid metabolism in these animals [33]. Thus, it can be hypothesized that several chemicals are likely to interact with testosterone metabolism in mysids, which as a result could lead to functional abnormalities.

We previously reported that alterations in testosterone and energy metabolism are sensitive endpoints in mysids exposed to different xenobiots [4,5,8]. Results from the present study corroborate this conclusion. Although the effects of methoprene on testosterone metabolism occurred at the same concentration as the effects on CEA, nonylphenol-induced alterations in testosterone metabolism were apparent at the lowest tested concentration, whereas no significant effects were observed in the CEA assay. Similarly, we previously observed significant effects of TBT on testosterone metabolism in N. integer at concentrations 10 times lower than those at which effects on energy metabolism were noted [4,5]. Clearly, these differences are a reflection of toxicant-specific interactions and need to be further examined with a more extensive list of compounds. The CEA assay has the advantage that it is inherently related with effects at higher levels of biological organization [18]. This correlation also should be investigated for testosterone metabolism in mysids after chronic exposures. Finally, the field applicability of these biomarkers should be validated in the future. Preliminary studies investigating testosterone and energy metabolism in field-exposed mysids from the Scheldt estuary are promising [8].

CONCLUSION

The present study indicates that energy and testosterone metabolism of mysids are sensitive endpoints that are able to detect endocrine-disruptive activity of chemicals after short-term exposure.

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