



## 1. Introduction

Lately, it has been demonstrated that a number of xenobiotics in the environment are capable of causing adverse effects in animals and humans by interfering with their endocrine functions at different modes of action. Some of these so-called endocrine disruptors, endocrine modulators or hormone-mimetic substances, have been reported to be associated with decreasing sperm counts in man, increased frequencies of sex-hormone dependent forms of cancer (breast, testis, prostate, etc.), genital abnormalities (e.g. hypospadias, cryptorchidism), premature puberty in females and increased occurrence of endometriosis in humans (Gist, 1998). Among the suspected substances, various phenolic compounds are listed. However, our knowledge about the effects of these substances, especially on invertebrates, is still rather limited.

Bisphenol A (BPA) is one of the chemicals with the highest production rate world-wide (Heemken et al., 2000). It is mainly used in the plastics industry as an intermediate in the production of polycarbonate and epoxy resins. A smaller amount is used as antioxidants in plastics and hydraulic liquids, for the manufacturing of the flame retardant tetrabromobisphenol A, for thermopaper in printers and faxes, for tyres and in dental composites and sealings (BUA, 1997; Heemken et al., 2000). In Germany, the total BPA consumption was approximately 163 000 t in 1994, and 210 000 t in 1995 (Leisewitz and Schwarz, 1997). The corresponding amounts in 1993 were 347 000 t for Western Europe, 552 000 t for the USA and 214 000 t for Japan (BUA, 1997). With log  $K_{OW}$  values of 2.20–3.82 (BUA, 1997; Staples et al., 1998; Heemken et al., 2000) and a solubility of 120 mg/l (Howard, 1990), a moderate adsorption to sediments and accumulation in organisms can be expected. In water, BPA is easily degraded with a half-life of 2.5–4 days (Dorn et al., 1987) or 28 days in sewage treatment plants (Howard, 1990). Nevertheless, BPA is continuously detected in aquatic ecosystems (Belfroid et al., 2002) and is regarded as not readily biodegradable in general (BUA, 1997).

Octylphenol (OP) and nonylphenol (NP) are among the most important high production volume alkylphenols. Nonylphenol ethoxylates account for approximately 80% of the world market and octylphenol ethoxylates represent the remaining 20% (White et al., 1994). Alkylphenol ethoxylates are utilised in various industrial processes, e.g. wool washing, but are no longer allowed in domestic detergents in the European Union. These substances are biodegraded by eliminating the ethoxy groups which yields the less biodegradable alkylphenols (such as NP and OP). These endure often through sewage treatment and in rivers (Ahel et al., 1994a,b). The annual consumption of alkylphenol ethoxylates was estimated to be 13 500 t in Germany in 1986 (BUA, 1991) and 18 500 t in the UK in 1992 (CES, 1993).

OP is mainly used as by-product in the production of non-ionic tensides, with an estimated annual production over 2000 t in the European Union in 1992 (Kaiser et al., 1998). In the UK, the total OP emission into waters was estimated to be about 300 kg in 1998 (Environment Agency, 1998). A log  $K_{OW}$  of 3.96–4.21, slightly higher than that of BPA, suggests moderate to high adsorption to sediments and accumulation in organisms (Ahel and Giger, 1993). Besides, OP shows a high  $K_{OC}$  value of 3500–18 500 ml/g (Johnson et al., 1998) and a rather low solubility in water of 12.6 mg/l (Ahel and Giger, 1993) which confirms this behaviour.

The annual production of 4-nonylphenol was 200 000 t world-wide in 1987 (Rippen, 1999). It is mainly used in the plastics industry as an intermediate in the production of softening agents and stabilisers, but also in fungicides, herbicides, paints and pharmaceuticals. The estimated annual emission of NP to the environment is 5000 t (Rippen, 1999). NP has a half-life of 29 days in water and showed a transformation of 80% in sediments in 70 days (BUA, 1991). In sewage sludge, NP seems rather persistent as no degradation was observed after 28 days (Klopman and Tu, 1997). With a log  $K_{OW}$  between 3.28 and 4.48 (BUA, 1991; Ahel and Giger, 1993) and a rather low solubility in water of 5.9 mg/l (Ahel and Giger, 1993), a moderate to high adsorption to sediments and accumulation in organisms can be expected. OP and NP are both

considered in the Priority Lists of Action of the European Commission (European Union, 2000).

There are only few studies that investigated the effects of xeno-estrogens on invertebrates. Whereas some invertebrate studies have been conducted which consider water exposure (Oehlmann et al., 2000; Schulte-Oehlmann et al., 2000), there are hardly any experiments looking at sediment exposure (Watts et al., 2001). In order to obtain more information on effects of the three substances in the sediment compartment, the so-called “memory of the water”, the application of a whole-sediment bioassay, with a sediment-dwelling organism as test species, is necessary—especially for the freshwater environment. Recently conducted studies recommend molluscs and particularly snails as most sensitive organisms concerning effects of endocrine disruptors in invertebrates (DeFur et al., 1999; Oehlmann et al., 2000; Schulte-Oehlmann et al., 2000; Duft et al., 2003).

## 2. Materials and methods

Our test species is the freshwater mudsnail *Potamopyrgus antipodarum* (Gastropoda, Prosobranchia, Hydrobiidae), which is native to Europe for over 150 years, yet a “newcomer” in European freshwater ecosystems. In the mid 19th century, it was introduced from New Zealand to Europe in ballast water of ships. In contrast to New Zealand populations, European populations consist almost exclusively of females whereas males are reported rarely (Wallace, 1979; Ponder, 1988). European *P. antipodarum* are parthenogenetic and ovoviviparous with shell heights reaching up to 6 mm. They inhabit the upper layers of aquatic sediments, feeding on plants and detritus.

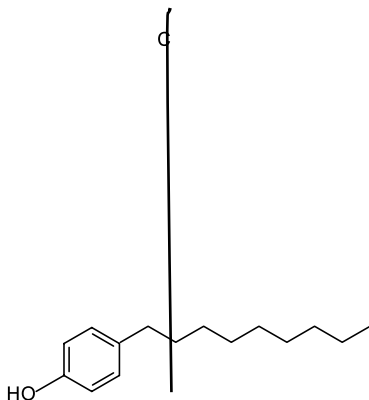
For all experiments, we used specimens from the breeding stock of our laboratory, which was built up with specimens collected from Gievenbach, a small creek near Ibbenbüren, Germany, in 2000. The snails were kept in 10-l aquaria in artificial freshwater (0.5 g NaHCO<sub>3</sub>, 5 g CaCO<sub>3</sub> and 5 g mineral salt per 10 l demineralised water, Milli Q RG and Milli RO plus, Millipore, Eschborn, Germany, pH 8.2±0.2) and fed regularly with a mixture of Tetra Phyll® (Tetra, Melle, Germany)

and Fish Tamin® (Sera, Heinsberg, Germany), stirred in the medium described above. Additionally, calcium carbonate was added regularly to improve shell growth.

The experimental design was set up according to Duft et al. (2003). Briefly, the experiments were conducted as static systems (without renewal of the water) in 1-l Erlenmeyer glass flasks. Artificial sediment consisting of 95% quartz sand (Quarwerke Millisil, Frechen, Germany) and 5% beech leaves (collected in the National Park on Rügen Island, Germany, crushed in a coffee grinder, MC 23, Siemens, Munich, Germany) was used for all exposures. The organic carbon content of this artificial sediment was 2.3%, the mean grain size was 180 µm. To each flask, 50 g of artificial sediment (dry weight) were added. For the spiking procedure, 2 ml of the respective concentration of BPA, OP and NP (dissolved in 100% ethanol) were applied to each treatment and homogenised by stirring. One-day evaporation guaranteed complete removal of the solvent. 1 l of medium was added to the flasks, which were subsequently aerated through glass pipettes (compressed air, 40 A compressor, Die Pumpe, Holm, Germany), enabling manual adjustment of air supply. Equilibration duration was 5 days in darkness. Finally, 80 *Potamopyrgus* individuals with a minimum shell height of 3.6 mm were added to each flask.

For bisphenol A (Merck Schuchardt Chemicals, Germany, >97% purity, batch no. 515 841 850), 4-*tert*-octylphenol (Merck Schuchardt Chemicals, Germany, >98% purity, batch no. 587 197 844) and 4-*n*-nonylphenol (Riedel-de-Haën, Germany, >99,6% purity, batch no. 0088x), see Fig. 1, the following concentrations were applied: 1, 10, 30, 100 and 300 µg/kg dry sediment (nominal). For nonylphenol, an additional concentration of 1000 µg/kg dry sediment was tested. Furthermore, a solvent control was included in each experiment, and as a positive control, one concentration of 17- $\alpha$ -ethinylestradiol (30 µg/kg dry sediment, Fluka, Germany, >85% purity, batch no. 315105/1 595) was included.

All tests were performed under constant conditions in a climate chamber with a temperature of 15±1 °C and a light:dark rhythm adjusted to 16:8 h. On  $t = 0, 2, 4$  and 8 weeks, 20 snails were taken



from each treatment and analysed individually (only 4 and 8 weeks for NP). Prior to analysis, the snails were narcotised in a 2.5%  $\text{MgCl}_2$  solution for 2 h. Shells were cracked with a small vice and shell parts removed. The brood pouch was opened carefully and the number of “grown-up” embryos (with shells) and “new” embryos (without shells) were counted under a dissecting microscope (Fig. 2). Additionally, the occurrence of egg cells in the oviduct and the maturity of the ovary were noted for each individual. Eventual mortality in the treatments was also registered; dead snails were counted and removed.

All data were analysed statistically using the software package PRISM<sup>®</sup>, Version 3.01 (Graph-Pad Software, San Diego, CA, USA). We calculated mean and standard error for each treatment and performed one-way-ANOVAs (analyses of variance), followed by Tukey multiple comparisons of the means to check for differences between the treatments and solvent control ( $P < 0.05$ ). For all substances, non-linear regressions were calculated using a sigmoidal model with a variable slope. By reparameterising the model, respective effect concentrations were calculated:  $\text{EC}_{10}$  (concentration of the substance causing a 10% increase of performance compared to the solvent control) and  $\text{EC}_{50}$  (concentration of the substance causing a 50% increase of performance compared to the solvent control).

### 3. Results

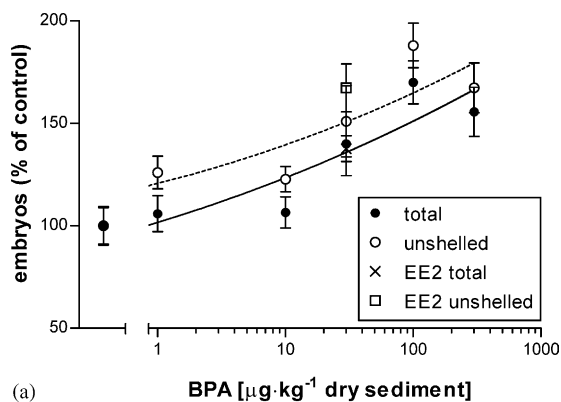
Exposure to bisphenol A and octylphenol resulted in a marked increase in the number of embryos in the brood pouch of *P. antipodarum*.

#### 3.1. Effects of bisphenol A

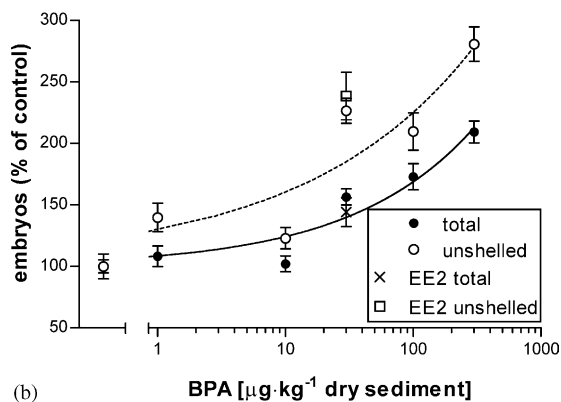
For BPA, a continuous increase in the number of unshelled embryos was noticed already after 2 weeks: All treatments (except for 1 and 10  $\mu\text{g}$  BPA/kg) showed a significant stimulation (up to 90% above the value for the solvent control) of the embryo production (ANOVA,  $P < 0.05$  for 30  $\mu\text{g}/\text{kg}$ ,  $P < 0.01$  for 100 and 300  $\mu\text{g}/\text{kg}$ ), thus the respective LOEC was equivalent to 30  $\mu\text{g}$  BPA/kg (Fig. 3a). The estimation of effect concentrations yielded even lower values: the  $\text{EC}_{50}$  turned out to be 24.5  $\mu\text{g}/\text{kg}$ , the  $\text{EC}_{10}$  was 0.22  $\mu\text{g}/\text{kg}$  (Table 1,  $r^2 = 0.83$ ). For the total number of embryos, the same trend was observed, whereas the number of shelled embryos was not affected (ANOVA,  $P > 0.05$ ). The estimated effect concentrations for the total embryo number were 88.1  $\mu\text{g}/\text{kg}$  ( $\text{EC}_{50}$ ) and 2.92  $\mu\text{g}/\text{kg}$  ( $\text{EC}_{10}$ ), respectively (Table 1,  $r^2 = 0.96$ ).

After 4 weeks of exposure, a more distinct increase of the embryo number was noticed (Fig. 3b). The production of “new”, unshelled embryos was significantly stimulated (ANOVA,  $P < 0.001$ )

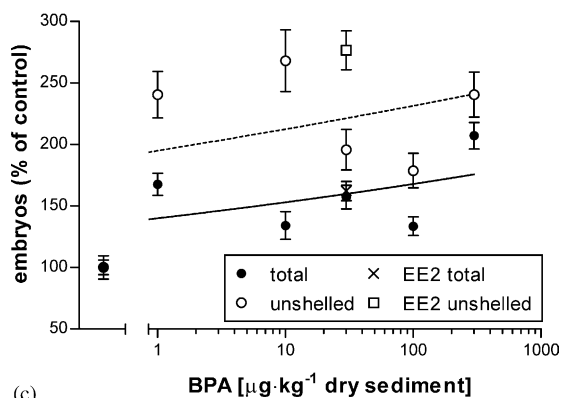
at 30, 100 and 300  $\mu\text{g}$  BPA/kg (up to 170% above the solvent control value). For the total number of embryos, a similar tendency can be seen (Fig. 3b).



(a)



(b)



(c)

Fig. 3

The estimated  $\text{EC}_{50}$  for the embryo production was  $5.67 \mu\text{g}/\text{kg}$  ( $\text{EC}_{10}$   $0.19 \mu\text{g}/\text{kg}$ ,  $r^2 = 0.86$ ) and  $37.7 \mu\text{g}/\text{kg}$  for the total embryo number ( $\text{EC}_{10}$   $6.33 \mu\text{g}/\text{kg}$ , Table 1,  $r^2 = 0.95$ ).

After 8 weeks, the increase of the embryo production was even more conspicuous (Fig. 3c): the number of unshelled embryos was significantly elevated in all tested concentrations of BPA compared with the control sediment (ANOVA,  $P < 0.001$ ,  $P < 0.01$  for  $30 \mu\text{g}$  BPA/kg,  $P < 0.05$  for  $100 \mu\text{g}$  BPA/kg), hence the LOEC was  $1 \mu\text{g}$  BPA/kg. The estimation of effect concentrations yielded even lower values: the  $\text{EC}_{50}$  turned out to be  $4 \text{ ng}/\text{kg}$  (Table 1,  $r^2 = 0.71$ ). BPA stimulated the embryo production up to 170% above the solvent control value in most exposure groups. Also the two lowest concentrations of BPA resulted in a stimulation of the embryo production. A sharp and significant (ANOVA,  $P < 0.01$ ,  $P < 0.05$  for 10 and  $100 \mu\text{g}$  BPA/kg) increase was also noted for the total number of embryos (Fig. 3c), the corresponding  $\text{EC}_{50}$  was  $10.9 \mu\text{g}/\text{kg}$  ( $\text{EC}_{10}$   $50 \text{ ng}/\text{kg}$ , Table 1,  $r^2 = 0.50$ ).

The solvent control had no effect on embryo production (ANOVA,  $P > 0.05$ ). The total and shelled embryo number in the control did not change during the exposure periods of 2, 4 and 8 weeks (mean total  $9.90 \pm 0.49$ , mean shelled  $5.86 \pm 0.45$ ; ANOVA,  $P > 0.05$ ). For the number of unshelled embryos, a slight but not significant decrease was noted in the course of the experiment ( $4.02 \pm 0.93$ , ANOVA,  $P > 0.05$ ). No significant mortality occurred during the experiments with BPA.

### 3.2. Effects of octylphenol

For OP, the same trend as described for BPA was observed. Already after 2 weeks, an increase in the number of unshelled embryos was noticed: All

Fig. 3. Effects of bisphenol A (BPA) concentrations in the sediment ( $\mu\text{g}/\text{kg}$ ) on embryo numbers (without shell and total) of *P. antipodarum* in percentage of the solvent control (mean  $\pm$  standard error of the mean [SEM],  $n = 20$ ), (a) after 2 weeks of exposure, (b) after 4 weeks of exposure, (c) after 8 weeks of exposure. Regression lines are added—solid line for the total embryo number, dotted line for the unshelled embryo number.

Table 1

Comparison of effect concentrations (LOEC, EC<sub>10</sub> and EC<sub>50</sub>, with 95% confidence interval [CI]) for bisphenol A (BPA), octylphenol (OP) and nonylphenol (NP) in µg/kg, for the analysed endpoints: embryo production (new embryos without shell) and total embryo numbers after 4 and 8 weeks

|     |                           | Embryo production |                  |            | Total embryo number |                    |                  |
|-----|---------------------------|-------------------|------------------|------------|---------------------|--------------------|------------------|
|     |                           | 2 weeks           | 4 weeks          | 8 weeks    | 2 weeks             | 4 weeks            | 8 weeks          |
| BPA | LOEC                      | 30                | 30               | 1          | 30                  | 30                 | 1                |
|     | EC <sub>10</sub> (95% CI) | 0.22 (0.14–0.35)  | 0.19 (0.16–0.21) | 0.001 (NC) | 2.92 (0.98–3.73)    | 6.33 (5.41–7.41)   | 0.05 (0.02–0.08) |
|     | EC <sub>50</sub> (95% CI) | 24.5 (9.29–64.7)  | 5.67 (5.45–5.89) | 0.004 (NC) | 88.1 (65.3–99.7)    | 37.7 (27.7–51.3)   | 10.9 (9.87–12.1) |
| OP  | LOEC                      | 1                 | 1                | 1          | NC                  | 30                 | 1                |
|     | EC <sub>10</sub> (95% CI) | NC                | 0.004 (NC)       | NC         | NC                  | 2.11 (0.89–2.91)   | NC               |
|     | EC <sub>50</sub> (95% CI) | NC                | 0.07 (0.02–0.12) | NC         | NC                  | 108.3 (85.2–127.4) | NC               |
| NP  | LOEC                      | –                 | 10               | 10         | –                   | 10                 | 10               |
|     | EC <sub>10</sub>          | –                 | NC               | NC         | –                   | NC                 | NC               |
|     | EC <sub>50</sub>          | –                 | NC               | NC         | –                   | NC                 | NC               |

NC, non calculable; –, not measured.

treatments (except for 10 µg OP/kg) showed a significant stimulation of the embryo production (ANOVA,  $P < 0.05$ ), thus the respective LOEC was equivalent to 1 µg OP/kg (Fig. 4a). For shelled embryos and the total number of embryos, no significant trend was observed.

After 4 weeks, the effect was more evident: The number of unshelled embryos increased continuously and significantly in all tested concentrations of OP (Fig. 4b) with up to 130% above the embryo number in the control sediment (ANOVA,  $P < 0.001$ ,  $P < 0.01$  for 1 and 10 µg/kg). The corresponding LOEC for the embryo production was 1 µg OP/kg and the estimated EC<sub>10</sub> yielded a very low concentration of 4 ng/kg (EC<sub>50</sub> 0.07 µg/kg, Table 1,  $r^2 = 0.71$ ). The total embryo number confirmed this tendency (Fig. 4b): The exposure groups of 30, 100 and 300 µg OP/kg differed significantly from the solvent control (ANOVA,  $P < 0.01$ ,  $P < 0.05$  for 30 µg/kg). The corresponding LOEC for the total embryo number was 30 µg OP/kg. Calculation of the EC<sub>10</sub> resulted in a value of 2.11 µg/kg (EC<sub>50</sub>: 108.3 µg/kg, Table 1,  $r^2 = 0.51$ ).

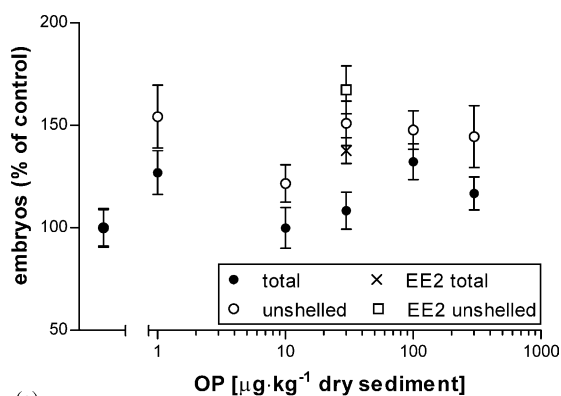
After 8 weeks, a typical inverted u-shaped concentration-response curve was observed: at higher concentrations of 100 and 300 µg OP/kg, the number of unshelled embryos was only slightly elevated (ANOVA,  $P < 0.001$  for 100 µg OP/kg,  $P > 0.05$  for 300 µg OP/kg). All other treatments

showed a highly significant stimulation of the embryo production with an increase of up to 150% compared with the control (ANOVA,  $P < 0.001$ , Fig. 4c), thus the LOEC was again 1 µg OP/kg. The calculation of effect concentrations ( $< 1$  µg/kg) was not possible. For the total embryo number, another trend was noticed. Again, for the lowest concentrations of OP, a sharp increase by 100% above the value of the solvent control was assessed (ANOVA,  $P < 0.001$ ), but already the treatments with 30 and 100 µg OP/kg went down to the solvent control level. The highest concentration of 300 µg/kg showed again a slightly higher embryo number than the solvent control (ANOVA,  $P < 0.05$ ).

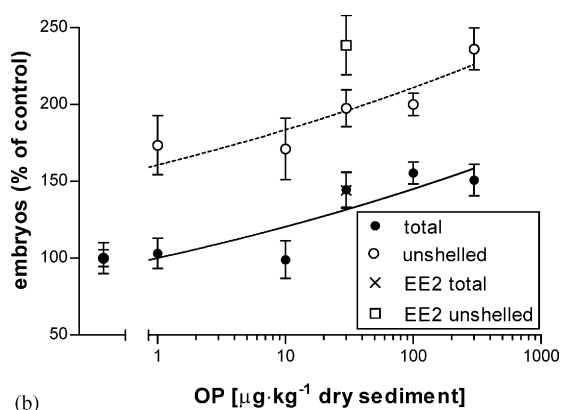
The solvent control had no effect on embryo production (ANOVA,  $P > 0.05$ ). No significant mortality occurred during the experiments with OP.

### 3.3. Effects of nonylphenol

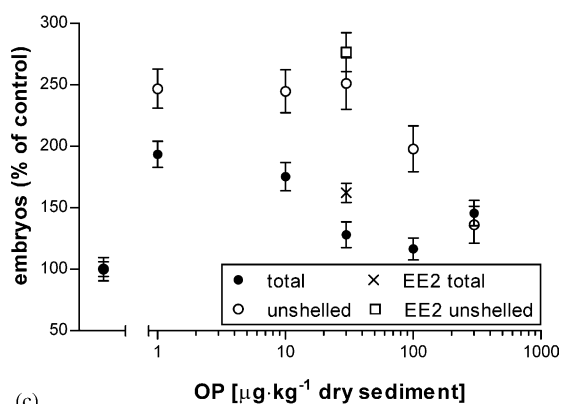
After 4 weeks of exposure, only the treatments with 10 and 30 µg NP/kg showed a slight, significant increase (up to 70% compared to the solvent control) in the total number of embryos (ANOVA,  $P < 0.05$ ) so that the LOEC for this experiment was 10 µg/kg (Fig. 5a). The same applies for the number of shelled and unshelled



(a)



(b)



(c)

Fig. 4

embryos. With the exception of 100 µg NP/kg, an inverted u-shape of the curve could be assumed.

After 8 weeks, however, a significantly elevated number of total and unshelled embryos was observed for 10 and 100 µg NP/kg (ANOVA,  $P < 0.05$ ), hence the LOEC was 10 µg NP/kg (Fig. 5b). 30 µg NP/kg and the higher concentrations of 300 and 1000 µg NP/kg resulted in clearly lower embryo numbers in comparison to the solvent control (significant for 1000 µg NP/kg, ANOVA,  $P < 0.05$ ). For NP, no effect concentrations could be estimated.

The solvent control had no effect on embryo production (ANOVA,  $P > 0.05$ ). No significant mortality occurred during the experiments with NP.

The positive control with ethinylestradiol (30 µg/kg) resulted in significantly higher embryo numbers in comparison to the solvent control for most exposure periods (ANOVA,  $P < 0.05$ ). After 2 weeks, an enhancement of the embryo production of 39% in comparison to the solvent control was found, while the total embryo number was not significantly increased (ANOVA,  $P > 0.05$ ; Fig. 3a, Fig. 4a, Fig. 5a). After 4 weeks, the embryo production was 140% higher than in the solvent control, and the total embryo number showed an increase of 44% (Fig. 3b, Fig. 4b, Fig. 5b). The increase of the embryo production after 8 weeks was 180%, and the total embryo number showed an increase of 63% (Fig. 3c, Fig. 4c, Fig. 5c).

### 3.4. Effect concentrations

A comparison of determined LOEC values and estimated effect concentrations of most of the investigated parameters in the experiments is given in Table 1. It is obvious that the number of newly produced embryos without shell was the most sensitive parameter for all tested compounds. It

Fig. 4. Effects of octylphenol (OP) concentrations in the sediment (µg/kg) on embryo numbers (without shell and total) of *P. antipodarum* in percentage of the solvent control (mean ± standard error of the mean [SEM],  $n = 20$ ), (a) after 2 weeks of exposure, (b) after 4 weeks of exposure, (c) after 8 weeks of exposure. Regression lines are added—solid line for the total embryo number, dotted line for the unshelled embryo number.

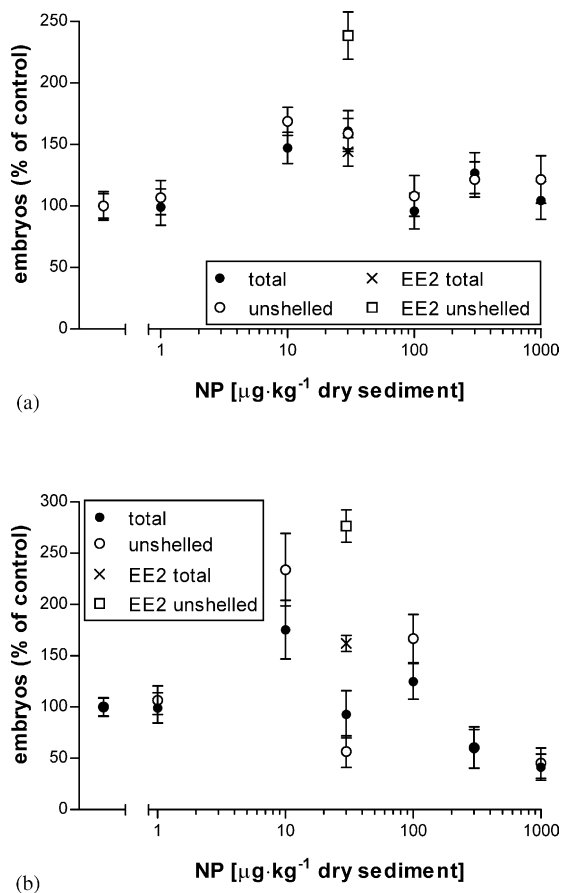


Fig. 5. Effects of nonylphenol concentrations in the sediment ( $\mu\text{g}/\text{kg}$ ) on embryo numbers (without shell and total) of *P. antipodarum* in percentage of the solvent control (mean  $\pm$  standard error of the mean [SEM],  $n = 20$ ), (a) after 4 weeks of exposure, (b) after 8 weeks of exposure.

should be noted that in general the  $\text{EC}_{50}$  values are more reliable than the  $\text{EC}_{10}$  values.

#### 4. Discussion

Analytical confirmation of the nominal sediment concentrations was not attempted because of the reported short half-lives of our substances in aqueous systems (Dorn et al., 1987; Schulte-Oehlmann et al., 2000; Kang and Kondo, 2002). Moreover, the analysis of sediments for these substances is quite complicated and for BPA, all

exposure concentrations lie below the reported detection limit of 5 mg/kg (Watts et al., 2001). Since accumulated concentrations in organism tissues generally best reflect observed effects, we tried to analyse the exposed snails' soft tissues, but this measurement failed because the sample weight (20 snails) was below the minimum required amount and hence, the necessary analytical sensitivity could not be achieved. However, in future experiments it would be advisable to measure the actual sediment concentrations when the appropriate (i.e. sensitive) analytical techniques are available.

Considering the above, it is likely that the sediment concentrations of the test substances decreased with time, not only because of degradation and desorption, but also due to uptake and possibly metabolism by the snails. Consequently, the actual exposure concentrations lie between the nominal values and zero. Thus, by evaluating the biological effect data on basis of nominal concentrations, it is actually a best-case scenario that is presented, since true effect concentrations are expected to be lower than those presented in Table 1.

The possibility that the test substances desorbed from the sediments or degraded during the experiments has to be considered when evaluating the results. Consequently, the embryo production of the snails is the net result of all effects (changes in availability, toxicodynamics and toxicokinetics) that occurred during the exposure period. By analysing the snails after 2, 4 and 8 weeks, we tried to describe the implications of these processes in a time-dependent manner on the embryo production.

In contrast to the previously mentioned studies, there are also reports on persistence of the test substances in sediments: Bettinetti et al. (2002) determined actual concentrations of 4-nonylphenol in spiked sediments which were about 20–25% less than nominal concentrations, but no concentration decrease was measured within 28 days. This is in agreement with the high persistence of 4-nonylphenol in sediments reported in both laboratory studies and littoral enclosures (Heinis et al., 1999).

#### 4.1. Effects of bisphenol A

It is difficult to compare our results of exposure to BPA via sediment with other results, as there are hardly any sediment studies in the literature. Some experiments with invertebrates were performed with an exposure via water, mostly using standard test organisms. Nevertheless, our results are in good accordance with other studies showing distinct adverse effects of bisphenol A on various organisms. The lowest effect concentration in another snail experiment is 1 µg/l: at this concentration, a complex “superfemale” syndrome was induced in the freshwater ramshorn snail *Marisa cornuarietis* and the marine gastropod *Nucella lapillus* in the presence of BPA (Oehlmann et al., 2000). Affected specimens were characterised by the formation of additional female organs, an enlargement of the accessory pallial sex glands, gross malformations of the pallial oviduct section resulting in an increased female mortality, and a massive stimulation of oocyte and spawning mass production. Besides, another (low-concentration) experiment with *M. cornuarietis* resulted in a LOEC of 48.3 ng/l and an EC<sub>10</sub> value of 13.9 ng/l for an increase of egg production and spawning (Schulte-Oehlmann et al., 2000). Exposure of *P. antipodarum* to BPA via water showed a LOEC of 5 µg BPA/l (Schulte-Oehlmann et al., 2000).

A LOEC of 20 µg/l was found for BPA-exposed copepods (Andersen et al., 1999a). Reduced moulting frequency was found in *Daphnia magna* (Zou and Fingerman, 1997), but these results could not be confirmed by Caspers (1998). In short-term in-vitro tests, BPA induced estrogenicity in 8 assays (Andersen et al., 1999b). Also, Lutz and Kloas (1999) report a binding affinity of BPA to the estrogen receptor of the amphibian *Xenopus laevis*. Besides, exposure of *X. laevis* to BPA during larval development resulted in a significantly elevated number of female phenotypes (Kloas et al., 1999). For other vertebrates, the following effect concentrations were found: at a feeding dose of 2 µg/kg body weight from gestation day 11–17, BPA reduced the daily sperm production in male offspring in mice (vom Saal et al., 1998). These results were not confirmed by Ashby et al. (1999). Also, increased post-natal

body weight gain and an advancement of puberty was found in female CF-1 mice whose mothers were treated with 2.4 µg BPA/kg from gestation day 11–17 (Howdeshell et al., 1999).

The most sensitive parameter in our experiments with *P. antipodarum* was the number of unshelled embryos, with a LOEC of 1 µg/kg after 8 weeks exposure and an EC<sub>50</sub> value of 0.2 ng/kg for this endpoint. To this value, the following actual environmental BPA concentrations may be compared: In small rivers near Berlin, up to 410 ng/l were measured in 1997 and in the river Elbe, concentrations between 17 and 776 ng/l were found (Heemken et al., 2000). The highest reported concentrations were measured in Japan near Tokyo with 10 ng/l up to 1.9 µg/l (Rippen 1999). In Elbe river sediments, 66–343 µg/kg were found (Heemken et al. 2000), and in Korean sediments, 54 µg/kg were determined (Khim et al., 2001).

It is apparent that the LOEC and EC<sub>10</sub>/EC<sub>50</sub> values for the increase of the embryo production in *P. antipodarum* (like in other molluscs, Oehlmann et al., 2000) are considerably lower than the actual and predicted environmental concentrations, indicating that the freshwater mudsnail and other prosobranch populations are endangered by the extensive utilisation of BPA.

Up to now, there is no limit value and no environmental legislative controls specific to BPA are known. In the EU, a tolerable daily intake (TDI) has been defined by the European Commissions Scientific Committee for Food (SCF) with a dose of 50 µg/kg body weight/day and a migration limit of BPA in food of 3 mg/kg was suggested. However, on the level of the European Union there is currently a risk assessment of BPA going on. The outcome of this assessment and the decision of this committee, with the United Kingdom as responsible country, will determine the further use of BPA in Europe.

#### 4.2. Effects of octylphenol

Analogous to BPA, it is difficult to relate our effects to other studies as experiments in sediments have rarely been performed. This deficit is explicitly obvious for freshwater sediments and fresh-

water organisms. In the fiddler crab, *Uca pugilator*, a reduced chitinase activity was found at an exposure to 10 mg OP/l for 7 days (Zou and Fingerman, 1999). Similar to BPA, Lutz and Kloas (1999) report a binding affinity of OP to the estrogen receptor of the amphibian *X. laevis*. Besides, exposure of *X. laevis* to OP during larval development resulted in a significantly elevated number of female phenotypes (Kloas et al., 1999).

Despite the voluntary self-control of industry and renunciation of alkylphenol ethoxylates in domestic detergents, OP is still ubiquitously present: In Swiss rivers, a broad range of alkylphenolic compounds was measured with concentrations of tens of  $\mu\text{g/l}$  (Ahel et al., 1994b). In the USA, low concentrations were found in drinking waters, the total of alkylphenols being 1  $\mu\text{g/l}$  with a concentration of octylphenol ethoxylate of 32 ng/l (Clark et al., 1992). In sediments, OP concentrations between 4 and 24000  $\mu\text{g/kg}$  were measured in lakes in the USA (Bennett and Metcalfe, 1998), and in rivers in Canada, 3–1,410  $\mu\text{g/kg}$  were detected (Bennett and Metcalfe, 1998). In German river sediments (Elbe), concentrations between 24 and 77  $\mu\text{g/kg}$  were measured (Heemken et al., 2000). In sewage sludge, even higher OP contents (22 mg/kg) were found (Bennett and Metcalfe, 1998).

Like for BPA, the most sensitive parameter in our experiments was the number of unshelled embryos. The LOEC for this endpoint after an exposure of 2, 4 and 8 weeks was 1  $\mu\text{g/kg}$ , the corresponding  $\text{EC}_{50}$  value after 4 weeks was 0.27  $\mu\text{g/kg}$  ( $\text{EC}_{10}$

(Khim et al., 2001). The comparison of laboratory effect concentrations for *Potamopyrgus* with NP residues in field sediments implies that at present, wild populations of this prosobranch species may face negative impact from this alkylphenol.

## 5. Conclusion

The xeno-estrogens BPA, OP and NP caused distinct increases in the embryo production and the total embryo number in *P. antipodarum* mostly already at the lowest applied concentrations. Evaluating the results for the tested substances, we can conclude that the chronic effects on *P. antipodarum* and their intensity are comparable and in good accordance with the experiments of Oehlmann et al. (2000) and Schulte-Oehlmann et al. (2000). In the presence of BPA and OP where we found sharper increases of the embryo numbers and lower effect concentrations (LOEC, EC<sub>10</sub> and EC<sub>50</sub>), the reproductive performance was stimulated to a greater extent than at exposure to NP. There was no acute toxicity in the conducted experiments.

Our results show that *P. antipodarum* is highly sensitive to the xeno-estrogens BPA, OP and NP in the sediment at environmentally relevant concentrations. Finally, it can be concluded that the sediment bioassay with *P. antipodarum* represents a promising system for the identification of endocrine disrupting substances and offers the more general possibility as tool for the assessment of sediment quality.

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