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Uptake, metabolism and excretion of bisphenol A in the rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The uptake, metabolism and excretion of the oestrogenic chemical bisphenol A (BPA) were studied in juvenile rainbow trout (*Oncorhynchus mykiss*). BPA was detectable in plasma, liver and muscle after 2 h of water exposure at 0.44 μM (100 μg BPA/l), and a steady state was reached within 12–24 h. The concentration of the glucuronidated degradation product in the plasma was about twice that of the parent compound. A plasma half life of BPA was calculated as 3.75 h following injection of the compound. The vitellogenin synthesis was measured in response to the BPA treatment, and a lag period of 5 and 7 days between injection of the compound and a significant vitellogenin response was observed for females and males, respectively. At the time of the vitellogenin response no BPA could be detected in the liver tissue from either male or female fish. These results indicate that fish briefly exposed to elevated levels of oestrogenic chemicals might develop a response several days later.

Keywords: Toxico-kinetics; *Oncorhynchus mykiss*; Bisphenol A; Bisphenol A glucuronide; Vitellogenin; Xenoestrogen

1. Introduction

For the past decades, an increasing effort has been made to elucidate the endocrine disrupting properties of natural as well as man-made compounds with hormonal activity (Toppari et al., 1996; Tyler et al., 1998). The endocrine disrupting chemicals (EDCs) can be found in a wide variety of matrices such as foodstuff, packaging materials and chemical formulations (Liehr et al., 1998). EDCs reach the aquatic environment mainly through sewage effluents thereby posing a potential threat to the organisms living in these ecosystems (Matthiessen and Sumpter, 1998). Field studies conducted on wild fish from English rivers show a high incidence of intersex among males living near sewage effluents (Jobling et al., 1998). Other experiments show that estrogenic responses can be detected in fish up to 5 km downstream from the site of sewage discharge (Harries et al., 1997). In addition to the estrogenic effects observed in natural aquatic environments, laboratory studies have revealed various endocrine

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disrupting properties of the compounds in question (Christiansen et al., 1998; Panter et al., 1998).

One of the compounds known to mimic the actions of natural oestrogen is bisphenol A (BPA) (Dodds and Lawson, 1936; Benjonathan and Steinmetz, 1998). In fish, an increased concentration of the yolk protein vitellogenin (VTG) has been observed following injection and water exposure of the compound (Christiansen et al., 2000; Lindholst et al., 2000). Apart from the endocrine disrupting effects observed for BPA and other hormonally active compounds, it is necessary to consider the uptake, metabolism and excretion in order to fully understand their modes of action. A rapid excretion or conversion of an oestrogenic compound into a less potent metabolite, might be a way in which an organism can reduce its oestrogenic load. Since EDCs often include phenols and other aromatic groups (e.g. alkyl phenols, phthalates, parabens and bisphenols) conjugation of polar moieties by phase II degradation enzymes is expected. The addition of a glucuronyl or sulfonyl group to the parent molecule is generally believed to increase the water solubility thereby facilitating the subsequent excretion (Giroud et al., 1998).

As regards the disposition of BPA, most of our present knowledge is based on mammalian studies. In 1966, Knaak and Sullivan examined the various routes of excretion of BPA in the rat, following oral exposure. They concluded that bisphenol A glucuronic acid (BPAGA) was a major degradation product subjected to renal excretion. Within recent years, the specific isoforms of glucuronosyl- and sulfonyl transferase, responsible for the enzymatic phase II conjugation of BPA have been characterised in the rat (Yokota et al., 1999; Suiko et al., 2000). In piscine systems, knowledge about metabolism and excretion of BPA, as well as most other EDCs, is scarce. Larsson et al. (1999) examined the presence of BPAGA in the bile from caged fish exposed to sewage effluent. Other studies measured the activity of phase II degradation enzymes in response to xenoestrogenic exposure (Andersson et al., 1985; Arukwe et al., 1997). However, the level of the BPAGA production as well as the excretion rate of BPA in fish has until now been unexplored.

The present study examines the toxicokinetic behaviour of BPA in the rainbow trout *Oncorhynchus mykiss*. The uptake, accumulation and excretion of nonmetabolised BPA was monitored in blood plasma and muscle- and liver tissue. Furthermore, the metabolism of BPA was estimated by direct measurements of BPAGA in the blood plasma. The quantification of degradation product was carried out by means of an enzymatically synthesised BPAGA standard. Finally the yolk protein vitellogenin (VTG) was measured in the plasma throughout the experimental period, in order to characterise the estrogenic response associated with BPA exposure.

2. Materials and methods

2.1. Experimental set-up

Juvenile rainbow trout (*O. mykiss*: 90–130 g) were purchased from a local fish farm, and acclimated for 10 days in 400-l tanks supplied with running fresh groundwater at 15°C. Fish were held in a 12-h light, 12-h dark photoperiod and were not fed during the experiment.

The experiment was carried out in a flow-through system consisting of 400-l tanks, fitted with circulatory pumps (Eheim 1250, Germany). Administration of water was controlled through the use of a centrifugal pump (UPS 25-40 Grundfos, Denmark) at a flow rate of 960 l/day, whereas BPA was administered through a peristaltic pump (Ole Dich Instrumentmakers, Denmark) at a flow rate of 60 ml/day.

One group containing 54 fish was continuously exposed to 100 μg BPA/l (Aldrich, Germany) dissolved in 96% ethanol while a control group, designated exposure control, containing 36 fish was administered 96% ethanol. In another group, 54 fish were injected intraperitoneally (i.p.) with a solution of 48% ethanol containing 35 mg BPA/ml giving a final tissue concentration of 35 mg BPA/kg. A group of 36 fish, designated injection control, was exposed to the vehicle alone.

At 0, 2, 6, 12, 24, 48, 72, 120 and 168 h, six fish from each of the exposure groups and four from each control group were anaesthetised in 0.02%

phenoxyethanol (Sigma, Germany) and a 1000 μl blood sample was obtained from the dorsal vein for vitellogenin, BPA and BPAGA analysis. Blood was collected into heparinised 1.5 ml Eppendorf tubes and centrifuged for 4 min at $4000 \times g$ in a Dich centrifuge. Plasma was pipetted into 1 ml Eppendorf tubes, and stored at −80°C until analysis. Subsequent to the sampling of blood, the liver was removed and finally a 2 g muscle sample was obtained from the thoracic region. All tissue samples were stored at −80°C.

2.2. Preparation of BPAGA and BPAGA-d_{16} standards

BPAGA and BPAGA-d_{16} standards were synthesised using a modified version of Stevenson and Hubl (1999) based on the enzymatic conjugation of BPA or BPA-d_{16} and UDP-β-D-glucuronic acid (UDPGA) by the liver microsomal enzyme UDP-glucuronyl transferase (UDPGT). Bovine liver UDPGT and UDPGA were purchased from Sigma, Denmark. The incubation buffer consisted of 50 mM Tris (pH 8.0), 1 mM dithiothreitol, 6 mM $CaCl_2$, 3% (w/v) bovine serum albumin and 8 mM UDPGA. UDPGT was added giving an enzyme activity of 0.044 U/ml. The reaction was started by the addition of BPA or BPA-d_{16} dissolved in the co-solvent acetonitrile giving substrate and co-solvent concentrations of 4 mM and 2% (v/v), respectively. The incubation was carried out at 35°C for 24 h.

2.3. Purification of BPAGA

Subsequent to incubation the reaction mixture was centrifuged at $15\,000 \times g$ for 15 min in a Sigma 3K30 centrifuge (Struers, Denmark) to remove suspended proteins. The supernatant was diluted with 4 vol. water and pH was adjusted to 2.0 with acetic acid (Merck, Germany). Sample amounts of 4 ml were applied on a Waters Sep-Pak C-18 500 mg cartridge previously conditioned with 5 ml methanol and 5 ml 0.2% formic acid. Before eluting the sample in 4 ml 65% methanol the cartridge was washed with 5 ml 0.2% formic acid and allowed to dry for 3 min.

The extracted material from the preceding sample clean-up procedure, dissolved in 65% methanol, was diluted into 20% methanol and made up with 0.2% formic acid before further purification via HPLC fractionating. Aliquots of 2 ml were applied to a 200×16 mm C-18 column pre-equilibrated with 0.2% formic acid. BPAGA or BPAGA-d_{16} was eluted with a linear gradient of 0–100% 0.2% formic acid in methanol for 40 min with a flow rate of 3 ml/min. The acquired HPLC fractions were pooled and evaporated to dryness by means of a vacuum dryer.

2.4. Characterisation of BPAGA

The purified BPAGA consisted of fine white needle-shaped crystals with a melting point of 150–154°C. BPAGA was dissolved in deuterated methanol (CD_3OD) (Merck, Germany) and analysed by NMR spectrometry. The ^{13}C NMR spectrum (500 MHz) was as follows (numbers of C-atoms relate to the molecular structure shown in Fig. 1): 161.1 (C-6), 144.6 (C-7), 144.0 (C-16), 134.8 (C-10), 131.0 (C-13), 116.8 (C-14), 116.6 (C-9), 105.4 (C-15), 103.7 (C-8), 90.6 (C-1), 65.4 (C-5), 64.5 (C-3), 62.6 (C-2), 61.1 (C-4), 30.7 (C-11), 19.5 (C-12). Further structural verification of the synthesized compound was obtained from a full scan mass spectrum ($m/z = 50$–450) using the same instrumental settings as described in Section 2.5, apart from an increased cone voltage of 150 V (Fig. 1).

2.5. Chromatographic parameters for BPAGA and BPAGA-d_{16}

A Hewlett Packard 1100 LC-MSD equipped with an electrospray ionisation (ESI) interface was used for separating, identifying and quantifying the actual concentrations of BPAGA and BPAGA-d_{16} in plasma. Chromatography was performed using a 150×2.1 mm Zorbax Eclipsed XDB reverse-phase HPLC column (5-μm particle size) equilibrated with 100% solvent A (0.2% formic acid). BPAGA and BPAGA-d_{16} were eluted for 2 min with solvent A followed by a linear gradient of 0–100% solvent B (100% methanol, 0.2% formic acid) for 20 min. The flow rate was

0.4 ml/min. The MSD was tuned automatically using the built-in calibrant delivery system. Selected ion monitoring (SIM) was performed in negative mode for the ions m/z 403 (BPAGA) and m/z 417 (BPAGA-d_{16}). The MS conditions were as follows: drying gas temperature 250°C, drying gas flow 10 l/min, nebulizing gas pressure 20 psig, capillary voltage 4000 V, fragmentor voltage 70 V. Peak area ratios of standards and samples with respect to internal standard were computed using Hewlett Packard Chemstation software. The concentrations of BPAGA in samples were determined by interpolation from the appropriate standard curve. The quantification limit for BPAGA using this method was 50 nM based on a 200-μl plasma sample. All chemicals used in the extractions and analytical procedures were of analytical grade.

2.6. Water and tissue analysis

Every second day two 20-ml water samples were collected from each tank. The water, liver and muscle samples were analysed for their content of BPA using microwave assisted solvent extraction (tissue samples only) and solid phase extraction followed by LC-MS analysis as described by Pedersen and Lindholst (1999). The limit of quantification for water samples was 2.2 nM based on a 20-ml sample whereas in tissue the limit of quantification was 219 nM (50 ng BPA/g) based on 1 g of fresh tissue.

2.7. Plasma analysis

Plasma samples were analysed for their content of BPA and BPAGA. Approximately 200 μl plasma were dissolved in 4 ml 0.2% formic acid (pH 2) and internal standards were added. For the quantification of BPA, 30 μl of a deuterated (BPA-d_{16}) internal standard was used, whereas 30 μl deuterated BPAGA (BPAGA-d_{16}) was used as internal standard for BPAGA. The samples were applied on a Waters Sep-Pak C-18 500 mg cartridge conditioned with 5 ml methanol and 5 ml 0.2% formic acid, and desorbed by 4 ml 65% methanol made up with 0.2% formic acid. Finally, the samples were evaporated to dryness in a vacuum dryer (Maxi dry lyo, Denmark) and redissolved in 300 μl 0.2% formic acid in methanol. Aliquots of 10 μl were used for analysis. The analysis of BPA was carried out using LC-MS equipped with an APCI interface as described by Pedersen and Lindholst (1999).

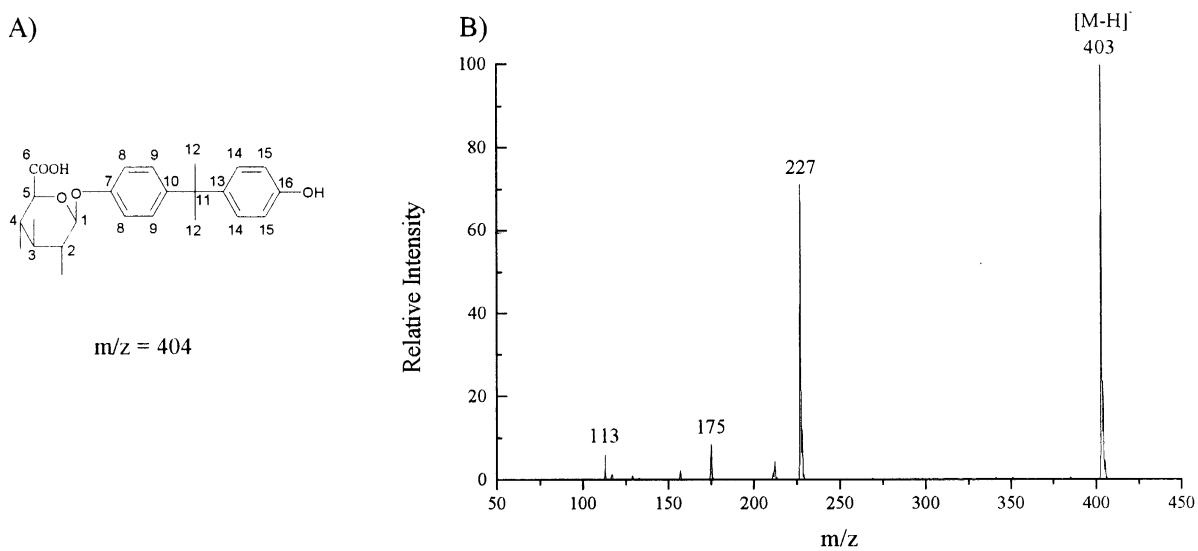

Fig. 1. Structure (a) and mass spectrum (b) of bisphenol A glucuronic acid (BPAGA) at a fragmentor voltage of 150 V. Numbers on the molecular structure of BPAGA refer to the interpretation of the ^{13}C NMR spectrum (see text).

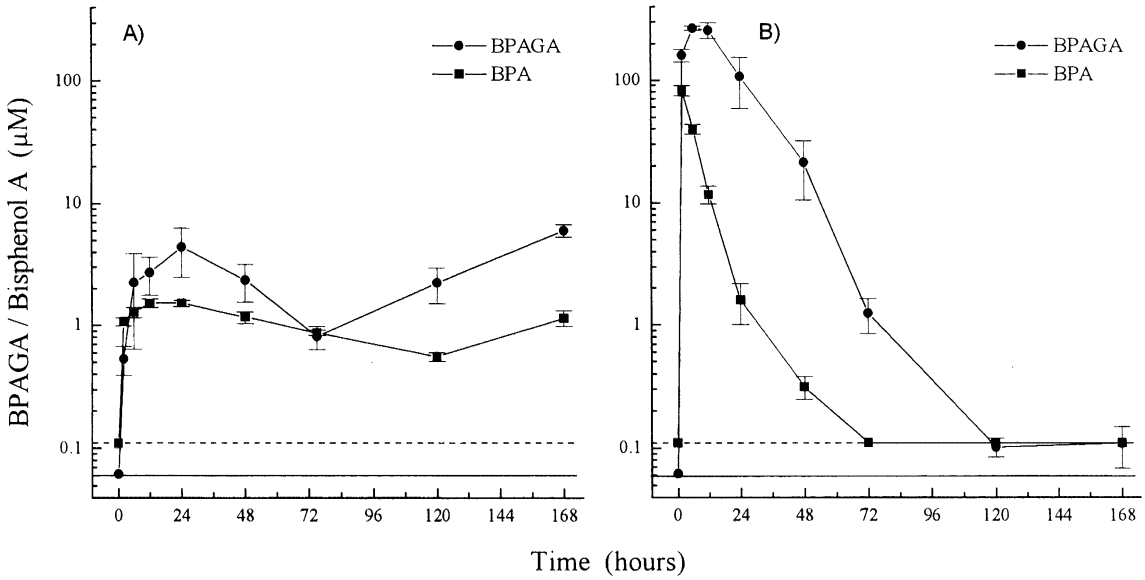

Fig. 2. Bisphenol A (BPA) and bisphenol A glucuronic acid (BPAGA) in the blood plasma of rainbow trout (mean ± SEM, $n = 6$). The fish were either water-exposed to 0.44 μM BPA (A) or intraperitoneally injected with 154 μmol BPA/kg (B). The dashed and solid horizontal lines indicate the limit of quantification for BPA and BPAGA, respectively.

2.8. Vitellogenin analysis

VTG in the plasma samples was measured by a direct sandwich ELISA based on polyclonal, affinity purified antibodies, as described in Christiansen et al. (2000). The present assay had a quantification limit of 0.04 μg VTG/ml.

2.9. Statistical analysis

Results obtained from the ELISA were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. All statistics were calculated using the software program Systat 7.0 with $P < 0.05$ as the level of significance.

3. Results

3.1. Characterisation of BPAGA

Following purification of BPAGA, a characterisation of the generated standard was carried out by ^{13}C NMR and mass spectrometry (Fig. 1). The mass spectrum of BPAGA recorded at a fragmentor voltage of 150 V, shows the $[M-H]^-$ ion at $m/z = 403$ and the $[M\text{-}177]^-$ ion at $m/z = 227$ originating from BPA. The fragments observed at $m/z = 175$ and 113 probably result from the glucuronic acid moiety.

3.2. Water, plasma and tissue levels of BPA and BPAGA

In one of the experimental groups, fish were exposed to a nominal concentration of 0.44 μM BPA (100 μg BPA/l). Every second day throughout the experimental period the actual water content of BPA was measured showing a mean concentration of 0.43 μM and a SEM of 0.004. No BPA could be detected in water from the injection group or any of the control groups.

Plasma samples, collected at 0, 2, 6, 12, 24, 48, 72, 120 and 168 from each of the two exposure groups were analysed for their content of BPA and BPAGA (Fig. 2). In the water-exposed group, an increase in BPA concentration was observed within the first 12 h reaching a maximum concentration of 1.5 μM (0.35 μg/ml). The same pattern

was seen for the appearance of the degradation product BPAGA in the plasma; concentrations reached a steady state in the region of 2.5–3.7 μM, approximately 2–2.5 times higher than the level of BPA. In the injection group, maximum concentrations of BPA and BPAGA of 83 and 265 μM were observed after 2 and 6 h, respectively. A decrease in BPA to levels below the limit of quantification was reached within 72 h. A first-order exponential decay regression line was calculated to $y = (\text{mean} \pm \text{SEM})e^{-(\text{mean} \pm \text{SEM})} = (118.5 \pm 1.7)e^{-(0.185 \pm 0.004)t}$ giving a BPA plasma half life of 3.75 h.

The internal doses of BPA in liver and muscle were measured throughout the 7 days of experimentation (Fig. 3). The concentration of BPA increased in liver and muscle to 2.4 and 1.7 μM after 24 and 48 h, respectively, in water-exposed fish. A decline at 72 and 120 h was observed in both liver and muscle to levels between 0.8 and 0.9 μM. In fish injected with BPA, maximum concentrations of 311 and 83 μM were reached within 2 h in liver and muscle, respectively. Concentrations dropped below the limit of quantification in liver after 168 h and in muscle after 120 h.

Excretion rates for BPA in plasma, liver and muscle were calculated from the data obtained in the injection group (Fig. 4). The BPA levels measured 2 h after injection were normalised to 100% giving comparable values on BPA excretion in the three compartments. The fastest excretion was observed in the liver with 19.4, 7.7 and 1.5% BPA remaining after 6, 12 and 24 h, respectively, whereas 48.9, 14.4 and 2.0% were remaining in the plasma. The lowest rate of excretion was, however, found in muscle where 73.8, 26.0 and 1.7% BPA remained after 6, 12 and 24 h, respectively.

Plasma samples collected from each of the two control and exposed groups were analysed for their content of VTG (Fig. 5). The straight lines shown in Fig. 5A,B represent mean VTG levels of all control male or female fish sampled throughout the experiment. The means ($\pm$SEM) for males and females are: 0.132 ($\pm$0.069) and 0.395 ($\pm$0.081) μg VTG/ml in the water-exposed group (Fig. 5A) and 0.108 ($\pm$0.026) and 0.464 ($\pm$0.136) μg VTG/ml in the injection group (Fig. 5B). A one-way ANOVA followed by Tukey's test for multiple comparisons was calculated for male and female fish from both exposure groups. Sig-

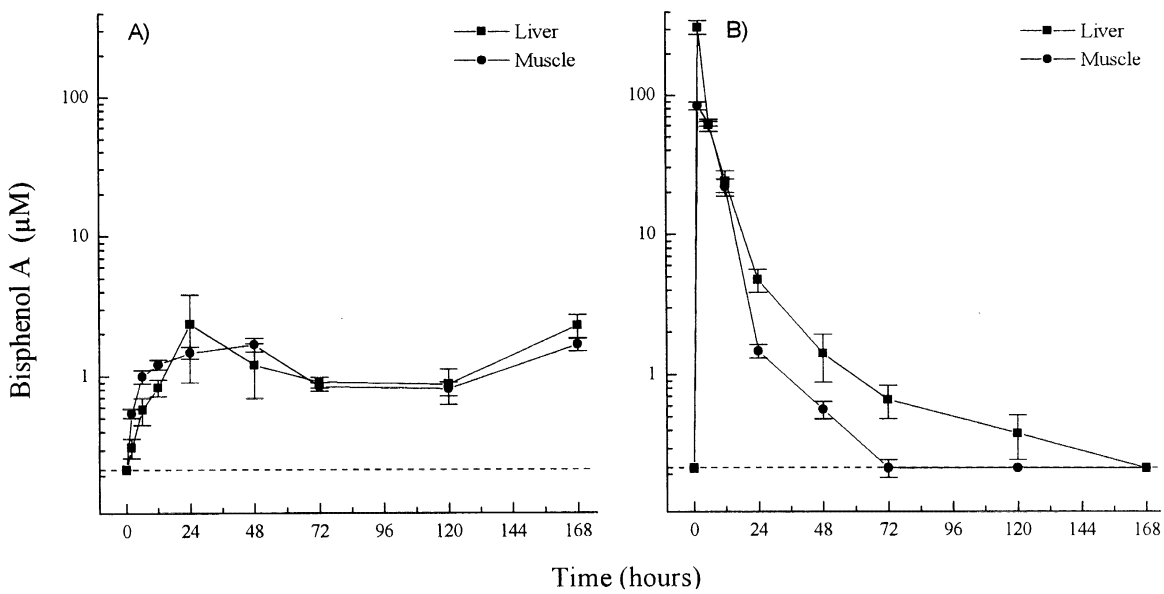

Fig. 3. Internal concentrations of bisphenol A (BPA) in liver and muscle tissue of rainbow trout (mean $\pm$ SEM, $n = 6$). The fish were either water-exposed to 0.44 μM BPA (A) or intraperitoneally injected with 154 μmol BPA/kg (B). The dashed lines indicate the limit of quantification.

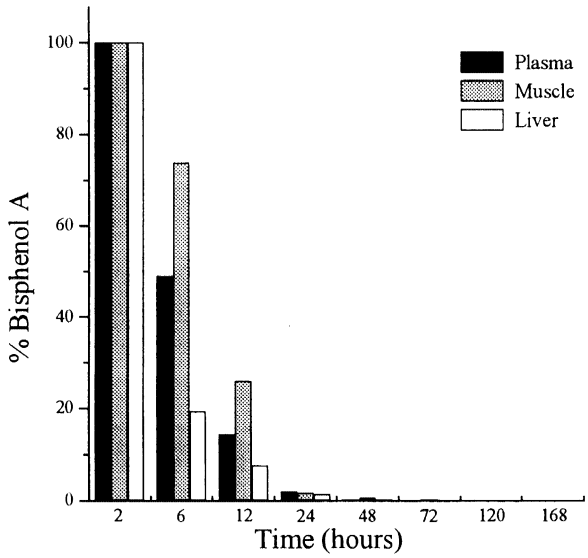

Fig. 4. A comparison of the bisphenol A (BPA) excretion from plasma, muscle- and liver tissue of rainbow trout injected with 154 μmol BPA/kg. The excretion rates are expressed as % BPA remaining relative to the levels observed at 2 h.

nificant differences in the VTG levels were found between the control group and female fish sampled at 120 and 168 h together with males sampled at 168 h in the injection study ($P < 0.05$). No significance was observed between fish from any of the water-exposed groups.

4. Discussion

The aim of the present study was to examine the uptake, accumulation, degradation and excretion of a well established xenooestrogen in a teleostean system. Two separate experiments, one on the water uptake and another on the excretion following compound injection were performed using the rainbow trout (*O. mykiss*) as a test organism. Compound administration in the water-exposed group was controlled by the use of a continuous flow-through system, assuring good agreement between nominal and actual concentrations. In the present study the actual water concentration of BPA only deviated by 2.3% from nominal values over a 7-day period.

When fish were exposed through the water, absorption of BPA reached a steady state level within 6–12 h in plasma, liver and muscle (Figs. 2 and 3). This uptake rate is in agreement with previous results reported for other phenolic compounds, e.g. a steady state was reached in 4 h in rainbow trout exposed to phenol (McKim et al., 1999). After reaching a steady state, bioconcentration factors (BCF) for BPA were calculated to approximately 3.5–5.5 for plasma, liver and muscle. For plasma, the BCF was calculated as (BPA/ml plasma)/(BPA/ml water) while BCF for liver and muscle tissue were calculated as (BPA/g tissue)/(BPA/ml water). We have previously reported BCFs at a BPA concentration of 100 μg/l to be 10.8 and 2.2 in liver and muscle, respectively (Lindholst et al., 2000).

The uptake of BPA in the blood plasma of rainbow trout coincides with the appearance of the glucuronidated degradation product BPAGA. Since the BPA molecule contains two phenol moieties, formation of conjugated degradation products such as sulfonated and glucuronidated metabolites are expected. Apart from the phase II metabolites generated from BPA, the introduction of an additional hydroxyl group to the parent molecule, leading to quinines, is an alternative degradation pathway observed in mammals (Atkinson and Roy, 1995). The formation of glucuronidated metabolites in response to BPA exposure was first described by Knaak and Sullivan (1966) in a mammalian system. The results from the present experiment show that the concentration of BPAGA increases to levels above BPA within 2–6 h. This suggests that glucuronidated products can be used as more sensitive indicators of xenobiotic exposure than the parent compounds. Previous studies suggest that BPAGA is accumulated in μg/g quantities in gall fluid from rainbow trout exposed to BPA (Larsson et al., 1999). One of the consequences of glucuronidation is a reduction in the partition coefficient (K_{ow}), meaning that compounds are made more water soluble and thereby easier to excrete. Giroud et al. (1998) showed that the introduction of a glucuronosyl group reduces the K_{ow} by two orders of magnitude. This would result in a reduction in the log K_{ow} for BPA from 3.4–3.8 to

approximately 1.4–1.8 for BPAGA (Staples et al., 1998). It is known that the activity of degradation enzymes can vary as a consequence of enzyme latency (Andersson et al., 1985). The lag period can either be ascribed to low activity of the existing enzymes or low levels of available enzyme. However, the rapid formation of BPAGA in the water-exposed group suggests that the conjugating enzyme, UDP glucuronosyl transferase, is already present in sufficient amounts to convert the absorbed BPA.

The excretion of BPA from plasma, liver and muscle was measured following an intraperitoneal injection of the compound. The fact that maximum concentrations of BPA are found in the three compartments 2 h after injection suggests that the compound is readily absorbed from the body cavity. The instant absorption of BPA into the circulatory system is important when the plasma half life is calculated, since compound constantly entering the blood stream would influence the result. The plasma half life of BPA was calculated to 3.75 h from a first-order exponential decay regression model ($r^2 = 0.924$). If the excretion rates in the three compartments examined are compared (Fig. 4) they can be ranked in the following order: liver > plasma > muscle. The fastest excretion rate is found in the liver where a reduction in BPA of 92.3% occurred within the first 12 h (relative to levels at 2 h). A somewhat smaller excretion rate is observed in muscle, which probably can be ascribed to the lack of metabolising enzymes. In the injection experiment, a lag period between maximum plasma concentrations of BPA and BPAGA is observed (Fig. 2B). This delay in BPAGA production could, as mentioned earlier, be ascribed to an increase in enzyme number or activity during the first 12 h.

In the present study, BPAGA quantification was performed by comparison of unknown samples to a synthesised standard. Since glucuronidated xenoestrogens are not commercially available, quantification of the compounds has so far relied on measurements of the parent compounds following cleavage by the enzyme β-glucuronidase (Kolanczyk et al., 1999; Nakagawa and Tayama, 2000). The direct measurement of glucuronide is however easier to perform and

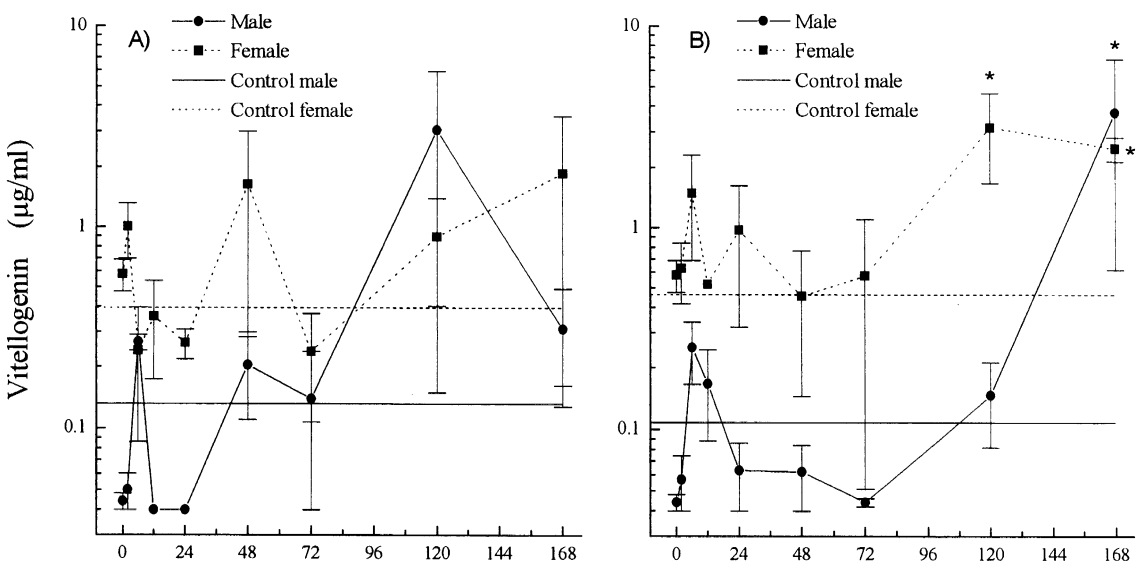

Fig. 5. Vitellogenin (VTG) concentrations in the blood plasma of male and female rainbow trout (mean ± SEM). The fish were either water-exposed to 0.44 µM BPA or intraperitoneally injection with 154 µmol BPA/kg. Group size varies between two and four individuals. The solid and dashed lines indicate mean VTG concentrations of control male or female fish sampled throughout the experiment. Asterisks indicate groups of fish significantly different from their respective control ($P < 0.05$). The limit of quantification was 40 ng VTG/ml.

involves fewer analytical steps that may influence the final result. Molecules containing two or more hydroxyl groups, like BPA, could be doubly conjugated by UDP-glucuronosyl transferase. In rats, the metabolism of brominated BPA results in 34% diglucuronidated conjugates to be found in the gall (Larsen et al., 2000). Diglucuronidated BPA was however not measured in this experiment.

The present study shows a lag period of 3–5 days for females and 5–7 days for male fish between BPA exposure through injection and a significant increase in the VTG concentration (Fig. 5B). During this lag period a possible generation of cellular components (e.g. the de novo synthesis of additional oestrogen receptors) necessary for the synthesis of vitellogenin may take place (Mommsen and Walsh, 1988). Le Guellec et al. (1988) were the first to describe this lag period in male rainbow trout following acute and chronic stimulation with estradiol. Though their results were not subjected to any statistical treatment it was evident that a several-fold induction in VTG levels occurred after 7 days of chronic exposure. An interesting aspect of the observed VTG response is the finding that BPA is almost absent from the liver at the time where maximal VTG levels are found in the blood (120 and 168 h). This result may suggest that the oestrogen receptors located in the hepatocytes may remain active for a certain time period after the release of the BPA molecules. As a consequence of this continual vitellogenesis, it is not possible to correlate actual internal BPA concentrations with VTG levels in a fluctuating environment. Furthermore, a sudden rise in the estrogenic load of polluted waters may cause an effect on the vitellogenesis several days later. This persistent vitellogenic response could reveal fluctuating estrogenic contents of polluted waters not observable from chemical analysis of water samples.

In the present experiment, male and female fish were divided into separate groups. This differentiation was necessary due to the fact that VTG levels differ between immature male and female fish (Carlson and Williams, 1999). Apart from the different control VTG levels, another sex difference is observed among fish from the injection group (Fig. 5B). The duration of the lag period before a significant VTG response is observed varies among males and females. In males, the response is detected at 168 h whereas in females significance can be seen at 120 h. Whether this difference is caused by an enhanced ability of juvenile females to induce vitellogenesis is uncertain. However, this observation further demonstrates that sex separation is necessary when the vitellogenesis is used as a biomarker.

One of the aims of this study was to monitor the metabolism of a xenoestrogenic compound. As a representative of the metabolites generated from BPA, the plasma level of BPAGA was measured throughout the experiment, showing concentration levels more than twice that of the parent compound. This finding, together with the fact that BPAGA is not the only degradation product generated from BPA suggests that the metabolism of xenoestrogens contributes to a significant reduction of the overall estrogenic load of the animal. The metabolism of xenoestrogens is however influenced by factors such as their distribution in the organism or availability in the circulatory system. Therefore further studies could reveal the actual significance of metabolism possibly explaining the relative potencies of various endocrine disrupting compounds.

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