INHIBITION OF TESTICULAR GROWTH IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) EXPOSED TO ESTROGENIC ALKYLPHENOLIC CHEMICALS

SUSAN JOBLING,*‡ DAVID SHEAHAN,‡ JULIA A. OSBORNE,‡ PETER MATTHIESSEN‡ and JOHN P. SUMPTER†
†Department of Biology and Biochemistry, Brunel University, Uxbridge, Middlesex, UB8 3PH, UK
‡Fisheries Laboratory, Directorate of Fisheries Research, Ministry of Agriculture, Fisheries and Food, Remembrance Avenue, Burnham on Crouch, Essex, CM0 8HA, UK
(Received 16 January 1995; Accepted 18 July 1995)

Abstract—It is becoming evident that an increasing number of widely used industrial and agricultural chemicals are estrogenic. The biodegradation products of a major group of nonionic surfactants, the alkylphenol polyethoxylates, are one such group. Some of these chemicals are widespread aquatic pollutants, and bioconcentrate in aquatic biota. Exposure of male rainbow trout (Oncorhynchus mykiss) to four different alkylphenolic chemicals caused synthesis of vitellogenin, a process normally dependent on endogenous estrogens, and a concomitant inhibition of testicular growth. The magnitude of these estrogenic effects was dependent on the estrogenic potency of the chemical, the stage of reproductive development of the fish, and the concentration of the chemical in the water. These results support the contention that exposure of wildlife to environmentally persistent estrogenic chemicals can result in deleterious reproductive consequences.

Keywords—Rainbow trout Oncorhynchus mykiss Alkylphenol Testicular growth Estrogen

INTRODUCTION

Recently, considerable concern has been expressed over the possibility that some man-made chemicals that mimic the effects of hormones (particularly estrogens) may adversely affect reproduction in wildlife and humans [1–4]. Like natural estrogens, these estrogenic chemicals are often small molecules that exert their action by binding to the estrogen receptor and regulating the activity of estrogen responsive genes. It has been known since the 1970s that some industrial chemicals exhibit estrogenic activity [5,6]. However, only recently has it been realized that many different groups of chemicals are estrogenic, including the relatively persistent degradation products of one class of nonionic surfactants [7,8] and some plasticizers [9]. These degradation products are ubiquitous contaminants in the aquatic environment; they also tend to bioaccumulate in animals.

Alkylphenols such as nonylphenol (NP) and related compounds are found widely in surface waters and aquatic sediments [10–14]. They are products of the microbial breakdown during sewage treatment of a group of industrial surfactants, the alkylphenol polyethoxylates (APEs) [15]. These are very effective nonionic surfactants, and as such, they are used not only in detergents, but also in paints, herbicides, and cosmetics. More than 300,000 tonnes of APEs are produced worldwide annually [16]. It has been estimated that 60% of this production ends up in the aquatic environment after sewage treatment as short chain alkylphenol polyethoxylates (e.g., nonylphenoldiethoxylate, NP2EO), alkylphenol carboxylic acids (e.g., nonylphenoxycarboxylic acid, NP1EC), and alkylphenols themselves (e.g., NP and octylphenol, OP) [15,17]. Environmental concentrations of individual alkylphenolic compounds are reasonably well documented but geographically variable. In general, the reported concentrations in sewage effluents and river waters refer almost exclusively to the alkylphenols NP and OP, although a few reports also mention the presence of the alkylphenol polyethoxylates [e.g., 11]. Like many of the other environmental estrogenic chemicals, alkylphenolic compounds are both strongly lipophilic and sorptive. Consequently, some of these chemicals have been reported to accumulate in animals [18,19] and sediments. Although the routes of exposure of these compounds to wildlife are probably diverse, exposure via contaminated water could be a major route, and hence any deleterious effects might be manifest to the greatest degree in aquatic animals such as fish and fish-eating birds and mammals.

We have previously demonstrated that not only NP but also some of the other biodegradation products of APEs (NP2EO, NP1EC, and OP), are estrogen to fish cells in vitro, although their relative potencies were approximately 100,000-fold less than that of the natural estrogen 17β-estradiol [8]. We have also tested representative APE degradation products in mammalian, avian, and fish in vitro estrogen assays and demonstrated that, at the molecular level, their action is mediated by the estrogen receptor and that this interaction is via a similar region of the hormone-binding domain as estradiol itself [20]. However, the physiological consequences of exposure of wildlife and humans to these compounds are not known.

The role of estrogens in the control of fish reproduction has been well studied in salmonid fishes, particularly in rainbow trout (Oncorhynchus mykiss) [21]. It is well established that natural estrogens (particularly 17β-estradiol) play a pivotal role in controlling reproduction of female rainbow trout, whereas in male fish, this process is controlled by androgens. In male rainbow trout, circulating levels of endogenous estradiol are undetectable, and hence probably very low. In other teleosts there is some evidence that male fish do synthesize small amounts of estradiol, and that the hormone may regulate the production of the male androgens 11-ketotestosterone and testosterone [22]. A major effect of exposure of male or female teleosts to estrogens is the stimulation of the synthesis and secretion of vitel-
logenin by the liver [23]. The sequestration of this protein by the growing oocytes in the ovaries of female rainbow trout is responsible for the enormous growth of the oocytes in the months prior to ovulation; plasma concentrations of vitellogenin undergo a million-fold increase during this period [24]. Although vitellogenin is generally a female-specific protein, male fish will produce this protein if they are exposed to estrogens, and therefore the presence of vitellogenin in the plasma of a male trout is a very sensitive biomarker of exposure to an estrogenic chemical.

Developing animals have been reported to be very sensitive to estrogens: in male rats, exposure to synthetic estrogens causes decreased testosterone production and morphological damage to the testes [25]. Exposure of pregnant female rats has been shown to have detrimental effects on fertility of both male and female offspring due to reproductive tract developmental abnormalities [26]. Similarly, estrogenic chemicals such as the pesticides DDT and methoxychlor have been reported to have detrimental effects on reproductive development in exposed animals. Treatment of gull eggs with o,p′-DDT, for example, resulted in feminization of male birds [27]; and the phallus of male alligators living in a lake contaminated with pesticides (some of which were estrogenic) was observed to be one-half to one-third the normal size [28]. As in mammals, estrogenic chemicals can be detrimental to fish; high doses of 17β-estradiol administered to juvenile rainbow trout caused kidney failure and death, probably as a result of excess vitellogenin accumulation [29]. Similarly, the estrogenic pesticide β-hexachlorocyclohexane can cause induction of vitellogenesis and hermaphroditism in juvenile guppies and medaka, respectively [30,31].

When taken together, the physiological effects of estrogenic chemicals on animals appear to be manifested primarily in the gonads. We therefore exposed male rainbow trout to measured concentrations of different alkylphenolic compounds and examined not only whether or not they were estrogenic, but also whether they affected testicular growth.

MATERIALS AND METHODS

Groups of 2-year-old adult male rainbow trout (n = 12–15) held in large glass tanks (500 L) were maintained in filtered (by reverse osmosis) borehole water in a continual flow-through system, either in the absence of chemical, or in the presence of various alkylphenolic chemicals, for periods of 3 weeks. The trout used for the three different experiments described below underwent exposure at three separate time points during their reproductive cycle (May, August, and November). In all three experiments, the fish were fed daily to satiation.

4-Nonylphenol (NP) was purchased from MTM (Lancashire, UK), 4-tert-octylphenol (OP) and 4-nonylphenoxycarboxylic acid (NP1EC) were purchased from Aldrich (Dorset, UK), and nonylphenoldiethoxylate (NP2EO) was donated by ICI (Cleveland, UK). The preparations of nonylphenol, nonylphenoxycarboxylic acid (NP1EC), 4-tert-octylphenol, nonylphenoldiethoxylate (NP2EO), 17α-ethynylestradiol and NP2EO were liquid/liquid extracted using 2 aliquots of 25 ml dichloromethane. NP1EC and OP were extracted using solid-phase extraction onto 500 mg C18(EC) IST Isolute columns (Jones Chromatography, Hengoed, Wales, UK), followed by elution with 5 ml dichloromethane. All samples were internally standardized using butylphenol in methanol. Spiked aliquots of the control water were extracted, using both methods, together with each set of samples, and these acted as relative response samples. A Perkin Elmer (Beaconsfield, Bucks, UK) ISS 101 injector (1–10 µl injection volume) was used for sample delivery onto a Phase Separations (Deeside, UK) Spherisorb 25 cm × 4.6 mm S5 ODS2 bonded phase column held at 30°C in a Jones Chromatography oven. A Perkin Elmer series 410 quaternary HPLC pump was employed to isocratically pump 80:20 methanol : water at 2 ml/min; this gave full resolution of all compounds in 15 min. Eluted compounds were detected by a Perkin Elmer LS-4 fluorescence spectrometer (Ex 235 nm, EM 295 nm).

Initial experiment

In this experiment, we examined the effects of administration of four different alkylphenolic compounds (Fig. 1) to clean, filtered borehole water containing groups of male rainbow trout (n = 15) undergoing sexual maturation, and compared these effects with those observed when similar groups of rainbow
trout were exposed to a low concentration of an extremely potent synthetic estrogen, 17α-ethynylestradiol (EE2), as a positive control. The EE2 was purchased from Sigma (Dorset, UK) and was 99.99% pure. This initial experiment was done in May, when the testes of the fish were beginning growth.

A nominal concentration of 30 µg/L of each of the alkylphenolic chemicals was administered to the water, as this was considered representative of the concentrations that can be found in sewage effluents and some rivers (see Discussion). EE2 was added at a much lower concentration of 2 ng/L, based on previous studies designed to assess its potency in fish [32].

In addition to measuring the concentrations of the alkylphenolic chemicals in the aquaria water, EE2 concentrations were monitored using a specific radioimmunoassay. Prior to immunoassay, the EE2 was extracted and concentrated from 1-L samples of water using Sep Pak® C18 minicolumns. Extraction efficiencies were 90–95%. An established radioimmunoassay was used for the analysis. The detection limit of this assay was approximately 20 pg/L.

Blood samples were taken from all fish both initially and at the end of a 3-week period of exposure. Blood plasma was assayed for vitellogenin content using an established homologous radioimmunoassay [33]. Gonads were removed, weighed to the nearest milligram, and their size expressed as a percentage of the total body weight (gonadosomatic index: GSI) in each case. A single group of fish was sacrificed at the onset of the experiment (initial control) to establish initial values for each of the variables measured.

The middle portion of one of the testes was fixed in Bouin’s for 18 h and then stored in 70% industrial methanol (IMS). After dehydration, the tissue was embedded in paraffin wax and several 5-µm sections were cut from each of three different positions along the tissue. After staining in Mayer’s hematoxylin, proportions of the different cell types in the sections were assessed using a previously established method, as modified by Scott and Sumpter [34]. Five germ cell stages were identified: spermatogonia A, spermatogonia B, spermatocyte A, spermatocyte B, and spermatid. The area of the section occupied by cysts containing these cells was expressed as a percentage of the total weight of the testis.

**Dose–response studies**

Two separate samples (n = 90–100) of adult male rainbow trout were collected from a local trout farm in August and November and each was divided into seven groups (n = 12–15). The fish were held in glass tanks and maintained in pure borehole water (final control) or pure borehole water containing either NP or OP at a range of concentrations. The target concentrations of NP or OP in each experiment were as follows: 0.5 µg/L, 1.32 µg/L, 3.5 µg/L, 9.3 µg/L, 24.5 µg/L, and 65 µg/L. Blood samples were taken from fish both initially and at the end of a 3-week period of exposure. Blood plasma was assayed for vitellogenin content using an established homologous radioimmunoassay [33]. The NP experiment was done in August, when testicular growth is well underway, and the OP experiment in November, when testes are essentially fully grown. Gonads were removed, weighed to the nearest milligram, and their size expressed as a percentage of the total body weight (GSI) in each case. A single group of fish was sacrificed at the onset of the experiment (initial control) to establish initial values for each of the variables measured.

**Statistical analyses**

In all experiments, statistical analysis of the data was performed using a STATSVIEW statistical computing package (Apple Computer, Inc., Cupertino, CA, USA). One-way analysis of variance was used to determine the effect of different treatments on both vitellogenin concentration and GSI. This was followed by Scheffe’s test for multiple comparisons. One-way analysis of variance was also used to determine the effect of different treatments on the percentage of the testes occupied by each of the five cell types and hence the developmental state of the testes.

In the dose–response studies, t tests were used to determine the significance of the differences between each treatment and the final control. Correlation analysis was carried out to examine the significance of the relationships between vitellogenin concentrations and GSI, and regression analysis was carried out to examine the significance of any relationships between chemical concentrations and GSI.

**RESULTS**

**Initial experiment**

Concentrations of each of the compounds (expressed as mean ± standard error) measured throughout the experiment were as follows: control = 0, EE2 = 1.79 ± 0.1 ng/L, OP = 38.52 ± 6.49 µg/L, NP = 36.81 ± 2.4 µg/L, NP1EC = 31.82 ± 6.50 µg/L, NP2EO = 38.3 ± 3.01 µg/L. The experiment demonstrated that all of the alkylphenolic compounds are estrogenic and caused very significant elevations (p < 0.001) in the concentrations of vitellogenin measured in the plasma of exposed fish (Fig. 2A). When placed in order of potency according to the magnitude of the response, OP, at a nominal concentration of 30 µg/L, was the most potent alkylphenolic compound; a million-fold increase in the concentration of vitellogenin in the plasma of fish exposed to this compound was observed. The potent synthetic estrogen EE2, at a concentration of 2 ng/L, caused an effect of a similar magnitude; whereas the concentrations of vitellogenin in the plasma of fish exposed to NP, NP2EO, and NP1EC were elevated less, yet still by 100- to 1,000-fold.

Although all of the fish survived and grew during the 3-week experiment (data not shown), these pronounced increases in plasma vitellogenin concentrations were in all cases accompanied by concomitant significant decreases in the rate of testicular growth (p < 0.05; Fig. 2B). The development of the testes in rainbow trout is a well-documented process, and the stages of spermatogenesis have been defined [21]. The rainbow trout reproduces annually, with the testes growing from 0.1% to 5% or 6% of the body weight during each reproductive cycle [34]. At the beginning of the 3-week experiment, the GSI was 0.2 (i.e., the testes were 0.2% of the body weight). After 3 weeks, the GSI of the control groups of fish had risen, as expected, to 0.9, reflecting the rapid growth rate of the testes during this period of gonadal development. Conversely, the most estrogenic alkylphenol, OP, inhibited this testicular growth by 50%, a similar degree of inhibition to that caused by EE2, albeit at a 10,000-fold greater concentration. All other chemicals inhibited the growth of the testes significantly, but to a lesser degree. The degree of inhibition of testicular growth appeared to be dependent on the estrogenic potencies of the chemicals, because there was a very significant negative relationship (r = 0.52, p = 0.0001, n = 105) between the estrogenic potencies...
Estrogenic chemicals inhibit testicular growth

Fig. 2. Effect of estrogenic alkylphenolic compounds (30 μg/L) and ethynylestradiol (EE2; 2 ng/L) on the synthesis of vitellogenin and testicular growth in male rainbow trout exposed for 3 weeks. Values shown are mean values of vitellogenin in blood plasma (A) and of gonadosomatic index (GSI) (B). The error bars represent the standard errors. * denotes a significant difference from final control values at p < 0.05.

Fig. 3. The correlation between estrogenic potency (as measured by plasma vitellogenin concentration) and testicular size in rainbow trout after exposure to the alkylphenolic chemicals OP, NP, NP2EO, NP1EC, or the synthetic estrogen EE2 (r = 0.52, n = 105, p = 0.0001). Both horizontal and vertical error bars are shown (except in the case of EE2 where the horizontal error bars are too small to be shown).

Dose–response studies

Concentrations of NP (expressed as mean ± standard error) measured throughout the experiment carried out in August were as follows: control = 0, 0.24 ± 0.14 μg/L, 1.06 ± 0.3 μg/L, 1.85 ± 0.73 μg/L, 5.02 ± 1.12 μg/L, 20.3 ± 5.09 μg/L, 54.3 ± 12.68 μg/L. Exposure of maturing rainbow trout to various concentrations of NP demonstrated a clear dose-related increase in vitellogenin production. The lowest concentration of NP required to induce a significant (p < 0.05) elevation of the plasma vitellogenin concentration was 20.3 μg/L. The next concentration tested, 54.3 μg/L, induced a pronounced effect; the vitellogenin concentration increased more than 10,000-fold, to more than 1 mg/mL (Fig. 5A). This estrogenic effect on vitellogenin synthesis was accompanied by a less pronounced dose-related inhibition of testicular growth (Fig. 5B); the overall effect was significant (r = 0.282, p = 0.01, n = 96) and the highest concentration of NP (54.3 μg/L) completely inhibited testicular growth during the experiment. It should be noted that only one fish died during the course of the experiment and that all groups of fish gained weight, although not significantly (data not shown).

Concentrations of OP (expressed as mean ± standard error) measured throughout the experiment carried out in December were as follows: control = 0, 0.3 ± 0.2 μg/L, 0.6 ± 0.2 μg/L, 1.6 ± 0.7 μg/L, 4.8 ± 0.9 μg/L, 14.6 ± 3.2 μg/L, 43.9 ± 5.4 μg/L. OP also induced a dose-related synthesis of vitellogenin in fully mature or regressing fish. The lowest dose of OP required to induce a significant effect was 4.8 μg/L, suggesting that OP was about five-fold more potent than NP (Fig. 6A). However, fish exposed to OP did not display any significant differences in gonadal size at any of the concentrations used (with the exception of 4.8 μg/L; p < 0.05) when compared to the control group of fish. Thus, in this experiment, there was no dose-related effect on the size of the testes. In fact, there was an apparent (but not significant) decrease in the GSI in all groups of fish, including the controls, relative to the initial size of the testes (Fig. 6B). In addition, there was an apparent, but
not significant, decrease in the mean weight of the fish in all groups (data not shown).

DISCUSSION

These results represent the first reported study on the estrogenic effects caused by exposure of any male animal to alkylphenolic compounds. The identification of the yolk precursor, vitellogenin, in the plasma of male fish provided confirmation that the chemicals were acting as estrogens; the vitellogenin gene is estrogen-responsive, and expression is dependent on the interaction of estrogen with estrogen receptors in the liver [23]. In male trout, the vitellogenin gene is normally silent [35], but exposure to exogenous estrogens will cause expression.

The effects of very low concentrations of the synthetic estrogen EE2 on vitellogenin synthesis in male trout were extremely pronounced; almost a million-fold elevation in the plasma concentration of this protein was observed after only 3-weeks exposure. All of the alkylphenolic compounds mimicked the effects of EE2 in causing the male rainbow trout to synthesize vitellogenin in large amounts (Fig. 2A). Placed in order of potency according to the magnitude of the response, OP > NP = NP2EO = NP1EC. We previously reported similar relative potencies based on in vitro studies [8], although the concentrations required to induce responses in the in vivo experiment reported here are two orders of magnitude lower than the concentrations required to elicit responses in vitro. We suggest that this disparity could be due to the bioaccumulative properties of these compounds and/or to metabolism of the chemicals by the fish into more active metabolites.

In sexually developing fish, the pronounced effects on vitellogenin synthesis caused by exposure to the various estrogenic chemicals were accompanied by concomitant significant decreases in the rate of growth of the testes (Fig. 2B). These effects on testicular growth were also significantly correlated with the order of estrogenic potency of the compounds; that is, the most estrogenic compounds had the greatest inhibitory effect on testicular growth. These findings add strength to the hypothesis that this phenomenon is caused by estrogens, although the mechanism of this inhibitory effect of estrogenic alkylphenolic compounds on testicular development is unknown. Experiments carried out later in the year on more mature (NP experiment) or regressing fish (OP experiment) demonstrated that the degree of inhibition of testicular growth in trout exposed to alkylphenolic chemicals is both dose-dependent and affected by the timing of exposure. Trout exposed to a range of concentrations of NP, toward the end of the annual gonadal growth phase, showed a dose-related, although less pronounced, inhibition of testicular growth (Fig. 5B). Furthermore, using a similar concentration range of OP, we were able to demonstrate that the inhibitory effects on testicular growth seen in sexually maturing fish were absent in mature or regressing fish (Fig. 6B). These studies are supported by those of Billard and colleagues [36] in which fully mature or regressing male trout exposed to high concentrations of natural estrogens suffered no ill effects (in terms of spermatogenetic inhibition).

We do not know the mechanism(s) underlying inhibition of testicular growth by estrogenic chemicals. It is possible that these chemicals exert their effects directly on the testis, possibly via the inhibition of androgen synthesis (androgens are required for spermatogenesis) [22]. It is equally possible that estrogenic chemicals inhibit spermatogenesis by acting at one or more levels in the cascade of hormones that regulate development of spermatogenesis.
Estrogenic chemicals inhibit testicular growth.

Fig. 5. The effects of different concentrations of nonylphenol on vitellogenin synthesis and testicular growth in rainbow trout exposed for 3 weeks. Values shown are mean values of vitellogenin in blood plasma (A) and of gonadosomatic index (GSI) (B). The error bars represent the standard errors. * denotes significant differences from control values at \( p < 0.05 \).

Fig. 6. The effects of different concentrations of octylphenol on vitellogenin synthesis and testicular growth in rainbow trout exposed for 3 weeks. Values shown are mean values of vitellogenin in blood plasma (A) and of gonadosomatic index (GSI) (B). The error bars represent the standard errors. * denotes significant differences from control values at \( p < 0.05 \).

The testes. For example, estrogens may inhibit gonadotropin-releasing hormone (GnRH) synthesis in the hypothalamus, and/or gonadotropin synthesis in the pituitary gland. Recent research has shown that at least one of the GnRH genes [37], and at least one of the gonadotropin genes [38,39], of fish contain estrogen-responsive elements (EREs), and therefore it is likely that the expression of these genes, and hence the synthesis of GnRH and gonadotropins, is controlled at least in part by estrogens.

Thus, although it is generally accepted that the principal stimuli for fish spermatogenesis are pituitary gonadotropins and testicular androgens, the specific role played by each individual hormone has not been clarified. Regardless of the mechanism of action, it is obvious that the gonads of developing male animals are very sensitive to estrogens. When administered to pregnant female rats, for example, they cause reduced testicular weight and lowered sperm counts in the male offspring [26]. In fish, it is well documented that 17β-estradiol can cause complete inhibition of gonadal development when given via the diet to maturing male salmonids [36].

In view of this information, perhaps the observed effects of ethynylestradiol on testicular development, when administered to the water, are not surprising. The most disturbing observation is that common aquatic pollutants that are weakly estrogenic can mimic these effects when present in the water at microgram per liter concentrations. Moreover, although we have examined only two indices of exposure to estrogens—vitellogenin concentration and testicular size—the possible effects of estrogens on male (and female) fish are almost endless due to the multitude of roles played by estrogens in normal physiology. These range from sex differentiation at the egg/embryo stage, to sexual maturation of the adults [40–42]. Estradiol also affects metabolism (as do many steroid hormones); these effects are not so well investigated, although it appears that estradiol stimulates both protein and lipid deposition, and hence growth [43,44]. The most likely process to be affected is reproduction, because estrogens are pivotal to successful reproduction, particularly in females where they are vital for oocyte growth, egg formation, and provision of the yolk for the developing embryo [45–48]. Indeed, effects of estrogenic chemicals on female fish are very likely to occur, because the concentrations of estrogen receptors in female fish are much higher than those found in males [49].

The relevance of these findings, in terms of the impact of contamination of the aquatic environment by alkylphenolic chemicals, will depend on their environmental concentrations. Domestic sewage effluents can contain up to hundreds of micrograms of alkylphenolic compounds per liter [50], whereas some industrial effluents, such as those originating from pulp mills and textile companies, can contain significantly higher...
concentrations [51]. Most of the alkylphenols produced are concentrated in the sewage sludge, whereas the more hydrophilic compounds, such as NP1EC and NP2EO, will be present primarily in the liquid effluent [15,52]. Recent studies at sewage works in Italy have reported secondary effluent concentrations of up to 4 µg NP/L, 27 µg NPEO/L, and 145 µg NPEC/L [53]. Concentrations of NP and (to a lesser extent) NPEOs in rivers have been reasonably well documented, whereas studies reporting river water concentrations of NPEC are few. In general, concentrations of NP rarely exceed 10 µg/L [11,51,54], although there are large variations in the concentrations reported, and in rivers that receive significant amounts of industrial effluents, concentrations may exceed 100 µg/L [55]. Due to its lower usage (72,000 tonnes per annum), concentrations of OP in water are generally lower than those of NP, although few workers have studied the presence of this compound, or the other metabolites of OPE. In addition, much of the OP and OP entering rivers would be expected to adsorb to the sediment, as these compounds are strongly hydrophobic; up to 13.1 mg NP/kg has been reported in river sediments [12,56].

Recent studies, examining the biodegradation of alkylphenolic surfactants, have shown that the more hydrophilic compounds, such as NPEC and NPEO, are much more resistant to biodegradation than was originally thought [52,57,58]. These chemicals may therefore be present in the environment for relatively long periods of time, although further studies on their concentrations in the environment are needed before any firm conclusions can be made. Two recent studies describe the alkylphenol carboxylic acids (e.g., NP1EC) as the most abundant of the three known groups of metabolites of alkylphenolic surfactants found in solution; the maximum reported concentration of just one example, NP1EC, in river waters is 45 µg/L [56], and the average concentration often exceeds 10 µg/L. Studies of estuaries also show that significant amounts may remain in both the sediment and the water column [59]. However, more research is needed before any firm conclusions can be reached regarding the behavior of these compounds in seawater.

It is clear from the above discussion that substantial amounts of alkylphenolic chemicals enter the aquatic environment, both from secondary wastewater discharge into rivers and the sea and from sewage sludge. Determining environmental exposures is very difficult, and may not even be particularly meaningful, as the concentration of estrogenic chemicals actually in the fish will depend on a multitude of factors, including bioavailability, biotransformation, and bioconcentration/bioaccumulation. Few attempts have been made to measure concentrations of these chemicals in organisms. As expected from the log Koc of NP, it has a tendency to bioconcentrate in animals. In fish, BCFs have been reported to be between 13 and 3,400 [18,19], although an average figure would appear to be approximately 300. NP2EO and NP1EC are slightly less lipophilic than NP [60], and hence the BCFs for these compounds would be expected to be somewhat lower. Alkylphenolic compounds with long ethoxylate chains are much more water soluble, and would not be expected to bioconcentrate to any significant degree. Tissue samples taken from animals in a river with a low level of NP contamination, 3.9 µg/L, contained 0.1 to 0.2 mg NP/kg [19].

In summary, the short-term studies described here have shown that the threshold concentration of NP, above which a significant elevation in vitellogenin synthesis is observed, is approximately 10 µg/L. Assuming that NP1EC and NP2EO are similarly potent, they would also be expected to have similar thresholds. The threshold concentration of OP is about 3 µg/L, although it seems impossible to draw any conclusions regarding the environmental impact of this chemical due to the paucity of information on its concentration in waters.

Based on these results, the concentrations of nonylphenolic chemicals that have been reported in rivers are in some cases high enough to stimulate vitellogenin synthesis. Effects on testicular growth are also possible, although probably dependent on the stage of testicular development of the fish when they are exposed to these chemicals. These findings may have profound implications for wild fish populations, but longer-term studies are needed to investigate these possibilities. It is important to remember that fish may spend long periods in water containing these chemicals, and although the effects on the testes reported here occurred at concentrations in excess of 30 µg/L, the fish were exposed for 3 weeks only; it is possible that effects may also be observed after long-term exposure to lower concentrations. Furthermore, we do not know whether other estrogenic chemicals that also contaminate the environment produce similar effects, or indeed whether a combination of several estrogenic chemicals may have an additive or even synergistic effect [61,62]. It has, however, already been established that sewage discharges (which contain alkylphenolic contaminants at µg/L concentrations) strongly stimulate vitellogenin synthesis in rainbow trout [63], and similar effects have recently been seen in some rivers downstream of discharges (J. Harries, personal communication).

It therefore seems likely that we have identified a new, and potentially very important, aquatic pollution problem. It should be emphasized that, prior to this report, there was no evidence that alkylphenolic chemicals (or any other estrogenic chemical) have had any effects on aquatic organisms, that can with certainty be ascribed to their estrogenic activity. However, it is equally true to say that few studies have explored this possibility. The presence of alkylphenolic chemicals in the aquatic environment, their persistence and hence accumulation, together with the effects on testicular growth and vitellogenin synthesis reported here, makes it likely that situations already exist where “estrogenic” effects are occurring with potentially deleterious consequences.

Acknowledgement—This work was funded by the UK Department of the Environment. The authors would like to thank David Back for the supply of the ingredients used in the EE2 radioimmunoassay.

REFERENCES

Estrogenic chemicals inhibit testicular growth


