Evaluation of the Aromatase Inhibitor Fadrozole in a Short-Term Reproduction Assay with the Fathead Minnow (Pimephales promelas)

Gerald T. Ankley,* 1 Michael D. Kahl,* Kathleen M. Jensen,* Michael W. Hornung,* Joseph J. Korte,*
Elizabeth A. Makynen,* and Richard L. Leino†

*U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, Minnesota 55804; †Department of Anatomy and Cell Biology, School of Medicine, University of Minnesota, Duluth, Minnesota 55812

Received November 8, 2001; accepted January 14, 2002

Cytochrome P450 aromatase (CYP19) is a key enzyme in vertebrate steroidogenesis, catalyzing the conversion of C19 androgens to C18 estrogens such as β-estradiol (E2). The objective of this study was to assess effects of the CYP19 inhibitor fadrozole on fathead minnow (Pimephales promelas) reproductive endocrinology and physiology in a short-term reproduction assay proposed for identifying specific classes of endocrine-disrupting chemicals. A concentration-dependent reduction in fecundity was observed in fish exposed for 21 days to water concentrations of fadrozole ranging from 2 to 50 μg/L. Consistent with the expected mechanism of action, there was a significant inhibition of brain aromatase activity in both male and female fathead minnows exposed to fadrozole. In females, this inhibition was accompanied by a concentration-dependent decrease in plasma E2 and vitellogenin concentrations; the latter observation is consistent with the fact that activation of the estrogen receptor by E2 initiates hepatic vitellogenin production in oviparous vertebrates. Histological assessment of ovaries from females exposed to fadrozole indicated a decrease in mature oocytes and an increase in preovulatory atretic follicles. Exposure of male fathead minnows to fadrozole significantly increased plasma concentrations of the androgens testosterone (T) and 11-ketotestosterone (KT) and resulted in a marked accumulation of sperm in the testes. Results of this study indicate that the proposed fathead minnow assay should effectively identify test chemicals as potential aromatase inhibitors, both in the context of their reproductive toxicity and the specific mechanism of action. These results also should be of utility in assessing the potential ecological risk of CYP19 inhibitors, in particular in the context of relating alterations in subcellular indicators of endocrine function (changes in steroids, proteins) to adverse consequences in the whole organism.

Key Words: aromatase inhibitor; endocrinology; fish; reproduction; steroids; vitellogenin.
function are assessed, including alterations in secondary sexual characteristics, gonadal condition (relative weight, histopathology), and plasma concentrations of sex steroids (E2, testosterone, 11-ketotestosterone) and vitellogenin (a precursor to egg yolk protein; Specker and Sullivan, 1994). The assay described by Ankley et al. (2001), as well as relatively similar short-term reproduction tests with the fathead minnow, have been used to assess the effects of EDCs representative of different MOAs. The greatest emphasis to date has been upon weak and strong agonists of the estrogen receptor (Ankley et al., 2001; Harries et al., 2000; Kramer et al., 1998; Miles-Richardson et al., 1999a,b), but there also has been some work concerning the effects of androgen receptor agonists and antagonists on fathead minnow reproduction/endocrinology (Ankley et al., 2001; Makynen et al., 2000). These studies have demonstrated that the assay can effectively identify and discriminate among these receptor-based MOAs. However, to support implementation of the test described by Ankley et al. (2001) as a method broadly suitable for identifying EDCs, evaluation of its performance with respect to inhibitors of steroidogenesis is critical.

The objective of this study was to evaluate the effects of the aromatase inhibitor fadrozole (4-(5,6,7,8-tetrahydromazao[1,5-al-pyridin-5-yl]benzonitrile monohydrochloride; CGS 16949A) on reproductive performance and endocrinology of the fathead minnow. Fadrozole is a reversible competitive inhibitor of CYP19 that has been shown to affect E2 biosynthesis in mammals, birds, and fish (Afonso et al., 1999, 2000; Elbrecht and Smith, 1992; Schieweck et al., 1988; Steele et al., 1987). The results of this study indicate that fadrozole effectively alters steroidogenesis in the fathead minnow, producing a suite of effects that should be diagnostic for identification of CYP19 inhibitors in tests with chemicals with unknown MOAs. Our results also highlight the need for a more thorough consideration of aromatase inhibitors as EDCs of ecological concern.

MATERIALS AND METHODS

Fadrozole was provided as a gift from Novartis, Inc. (Dr. H. Cooper Eckhardt, Summit, NJ). Stock solutions of the fadrozole (~4 mg/l) were prepared by adding 63 mg of the neat chemical to 18 l of test water in a glass, slow-stir saturator system (U.S. EPA, 2001). Target concentrations in the test system were generated by combining solution from the saturator with clean dilution water, such that a continuous flow rate of 45 ml/min to the test tank was achieved. As solution was removed from the saturator, clean water was introduced, so that equilibrium concentrations of the fadrozole stock were maintained. Under the conditions of our delivery system/dosing regimen, approximately 0.4 g of fadrozole were utilized for the 21-day experiment. Filtered (1 µm) water from Lake Superior was used for the testing; the mean (range) water quality characteristics over the course of the assay were pH, 7.43 (7.02–7.82) hardness, 42.8 (39.5–45.0) mg/l as CaCO3; alkalinity, 40.6 (38.0–43.5) mg/l as CaCO3; and dissolved oxygen, 6.35 (4.66–7.29) mg/l.

Exposures were conducted using the general experimental design and techniques described by Ankley et al. (2001). Based on the results of an initial range-finding study (data not shown), the 21-day reproduction test was conducted at target fadrozole concentrations of 2, 10, and 50 µg/l. There were three replicates at each treatment level, plus a clean-water control for a total of 12 tanks. Each tank contained four female and two male adult fathead minnows from an on-site culture at the Duluth EPA laboratory. The fish were maintained at 25 ± 1°C under a 16:8 h L:D photoperiod and fed adult brine shrimp twice/day. Animals were monitored for 3 weeks prior to initiation of chemical exposure to provide tank-specific baseline data. Survival, general behavior, and fecundity were evaluated daily. A similar routine continued after starting the chemical exposure; in addition, all eggs were examined microscopically to determine fertility, and a subset of spawns from each of the treatment tanks was maintained in clean water for 5 days to determine hatching success.

After 21 days of chemical exposure, fish were removed from the test tanks and anesthetized with MS-222 (100 mg/l buffered with 200 mg NaHCO3/l), and blood was collected from the caudal artery/vein with a heparinized microhematocrit tube. Plasma was isolated by centrifugation and stored with aprotinin (0.13 units) at –80°C until determination of vitellogenin and sex steroids. Fish and gonads were weighed for determination of the gonadosomatic index (GSI), and one gonad from each fish was preserved in 1% glutaraldehyde/4% formaldehyde in 0.1 M phosphate buffer for histological analysis. Brains were removed from the fish, flash-frozen in liquid nitrogen, and stored at –80°C for subsequent determination of aromatase activity.

For histological examination of gonads, tissues were dehydrated in graded ethanol solutions and embedded in JB-4 methacrylate. Gonads from four males and eight females were examined from each of the control and the 2 µg/l treatment groups, while samples from two males and four females were evaluated at the two higher fadrozole concentrations. Longitudinally embedded gonads were sectioned at 2 to 3 µm in a step-wise fashion and stained with hematoxylin and eosin. For each ovary, three slides were made with one section from 500 µm deep into the organ and two sections from 1000 µm deep. Testes were sectioned in a similar manner, except that the sections were taken at 250 and 500 µm depths. Ovarian maturity was evaluated with respect to oocytes in the following stages: (1) primary growth, (2) cortical alveolus, (3) early vitellogenic, (4) late vitellogenic, and (5) mature/spawning oocyte (Leino and McCormick, 1997; Selman and Wallace, 1986). In addition to assessing the overall stage of the ovary, the number of preovulatory atretic follicles per section was determined. Testicular staging conformed to Goodall et al. (1987) and Leino et al. (1990): (1) spermatoocyte, (2) spermatid, (3) some sperm in lumen of seminiferous tubules, small lumina, and (4) plentiful sperm in lumen of seminiferous tubules, large lumina.

Vitellogenin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) with a polyclonal antibody to fathead minnow vitellogenin (Korte et al., 2000; Parks et al., 1999). Plasma E2 and testosterone (T) in both sexes, and 11-ketotestosterone (KT) in males were measured using radioimmunoassay (RIA) techniques adapted to small-volume samples (Jensen et al., 2001; U.S. EPA, 2001).

Aromatase activity is not one of the “core” endpoints in the EDC assay described by Ankley et al. (2001); however, given the expected MOA of fadrozole, activity of the enzyme in brains of male and female fathead minnows was determined in the present study. Brains were utilized as opposed to ovaries, because preliminary experiments with ovarian microsomes from individual fathead minnows failed to produce activity above background due to the small amount of recovered microsomal protein. Although there is evidence that different genes encode brain and ovarian CYP19 in fish (Chiang et al., 2001; Kishida and Callard, 2001; Tchoudakova and Callard, 1998), activities of both isoforms seemingly respond in a qualitatively similar manner to a variety of inhibitors (Zhao et al., 2001). In addition, a recent study with the fathead minnow suggests significant structural homology between brain and ovarian aromatase mRNA (Halm et al., 2001). Aromatase activity was determined by the enzymatic conversion of androstenedione to estradiol, with the release of 1H from the C-1 carbon and subsequent formation of tritiated water (Thompson and Siiteri, 1974). A modification of the assay of Melo et al. (1999) was used. Briefly, dissected brains from individual fish (10–20 mg wet weight of tissue per fish) were thawed, homogenized in 10 µl phosphate buffer (10 mM K2HPO4, 100 mM KCl, 1 mM EDTA, 1 mM DTT, pH 7.4) per mg brain tissue.
tissue, and centrifuged at 10,000 g for 10 min. Forty to 50 μl of supernatant was incubated in phosphate buffer with 4 nM (1,4,6,7-3H)-androstenedione (Amersham Pharmacia Biochem, Piscataway, NJ; specific activity 100 Ci/mmol) and 1 mM NADPH at 24°C for 3 h. Following the incubation, samples were placed in an ice bath, 150 μl of ethyl ether was added, and samples were held on ice for 10 min. Samples were then held at –80°C for 10 min to freeze the aqueous fraction. The ether fraction was discarded, and 300 μl of 5% dextran-coated charcoal (Sigma, St. Louis, MO) slurry was added to each tube and placed on ice for 30 min to remove any remaining steroids. Samples were centrifuged at 1500 g at 4°C for 20 min. A 300-μl aliquot of the supernatant was added to 5 ml of scintillation cocktail (Ultima Gold; Packard, Downers Grove, IL) and 3H was determined as dpm, using a Packard 2500-TR liquid scintillation counter. Data were corrected for background through analysis of samples that had been treated similarly in all respects, except they had been heated for 15 min at 90°C. Protein was determined in 5 μl samples with Bradford reagent (Sigma) and quantified by comparison to a standard curve generated with bovine serum albumin (Sigma).

Fadrozole concentrations were determined twice weekly in water from the saturator column and from each of the treatment tanks, using high-pressure liquid chromatography (HPLC) with diode-array detection (Yamagami et al., 1993). Water samples (~2 ml) were collected, placed in glass vials, and directly injected as 700-μl aliquots onto a 15-cm Adsorbosphere HS column (Alttech, Deerefi, IL) on a Hewlett-Packard 1100 HPLC with diode array detection at 230 nm. Samples were chromatographed using a gradient program with a mobile phase starting at 60% methanol/40% 50 mM phosphate buffer (pH 7.0) and increasing at 1 ml/min to 80% methanol/20% buffer. The external method of quantitation was used, with a five-point standard curve. A Lake Grove, IL) and 3H was determined as dpm, using a Packard 2500-TR liquid scintillation counter. Data were corrected for background through analysis of samples that had been treated similarly in all respects, except they had been heated for 15 min at 90°C. Protein was determined in 5 μl samples with Bradford reagent (Sigma) and quantified by comparison to a standard curve generated with bovine serum albumin (Sigma).

For most measurement endpoints, among-treatment differences (based on tank mean values) were assessed using ANOVA followed by Dunnett’s procedure (U.S. EPA, 2001). Aromatase activity was analyzed using t-tests to compare males to females within treatments (on an individual basis), and tank mean values between the control versus 50-μg/l treatment groups. When necessary, data were transformed for normalization and to reduce heterogeneity of variance. Computations were performed with Systat 7.0 (SPSS, Chicago, IL). Differences were considered significant at p ≤ 0.05.

RESULTS

No mortality of fadrozole-exposed or control fish occurred. Water concentrations of fadrozole were stable and relatively close to the target values of 2, 10, and 50 μg/l; the respective mean (SEM, n = 6) measured concentrations were 1.4 ± 0.02, 7.3 ± 0.42, and 57 ± 0.06 μg/l over the course of the 21-day test. Among-tank variability in a given treatment was small, with coefficients of variation (averaged across the duration of the test) of 5.4, 11, and 0.16%, respectively, for the 2, 10, and 50 μg/l treatments. There was no detectable fadrozole in the control water.

Brain aromatase activity was significantly reduced in both male and female fathead minnows exposed to 50 μg fadrozole/l (Fig. 1). The aromatase activity in control females was slightly higher than in control males; however, there was no significant difference in aromatase activity between the sexes within the 50-μg/l fadrozole treatment group (Fig. 1). Measurements of brain aromatase activity were not made in the 2- and 10-μg/l treatment groups.

Fadrozole caused a concentration-dependent decrease in plasma E2 and vitellogenin concentrations in the female fathead minnow (Figs. 2A and 2B). The aromatase inhibitor did not affect T concentrations in the females (Fig. 2C); however, exposure to fadrozole significantly increased concentrations of both T and KT in male fathead minnows (Figs. 3A and 3B). Vitellogenin and E2 concentrations in male fathead minnows were near the limit of detection and unaffected by exposure to fadrozole (data not shown).

Fadrozole caused no significant change in GSI of the female fathead minnows (Fig. 2D); however, there were marked effects on the maturity of the ovaries, particularly from fish in the 10 and 50 μg/l treatments. These ovaries were less mature (all stage 3) than those from the controls, which were either stage 4 or recently spawned (Figs. 4A and 4B). There was no histological indication (i.e., postovulatory follicles) that spawning had occurred in the two highest treatment groups, and evidence that only one of eight females examined had recently spawned at the lowest test concentration. An increase in the number of preovulatory atretic follicles also was observed in females from all three fadrozole treatment groups (Fig. 4B); the mean number of these follicles in sections from the control, 2, 10, and 50 μg/l treatments were 3.4, 11.1, 11.0, and 21.5, respectively.

There was a concentration-dependent increase in the GSI of male fathead minnows exposed to fadrozole (Fig. 3C). Histologically, this increase was correlated with a marked enlargement of the lumina of the seminiferous tubules, which were
filled with sperm (Figs. 5A and 5B); this enlargement was particularly prevalent in males from the two highest test concentrations.

There were no discernable alterations in external morphology, including secondary sexual characteristics, of fish exposed to fadrozole. There were significant effects of fadrozole on fecundity of the fathead minnow (Fig. 6). Mean (SEM) fecundity rate of the fish over the course of the 21-day exposure to 0, 2, 10, or 50 μg fadrozole/l was, respectively, $20.5 \pm 5.7$, $8.9 \pm 3.1$, $1.5 \pm 1.4$, and $1.2 \pm 0.2$.

**FIG. 2.** Effects of fadrozole exposure on reproductive endocrinology of female fathead minnows: plasma concentrations of (A) β-estradiol (E2), (B) vitellogenin (Vtg), (C) testosterone (T), and (D) the gonadosomatic index (GSI). Data are mean (SEM) for three replicates at each of three fadrozole concentrations (μg/l) and the control. Asterisks (*) indicate significant difference from the control.

**FIG. 3.** Effects of fadrozole exposure on reproductive endocrinology of male fathead minnows: plasma concentrations of (A) testosterone (T) and (B) 11-ketotestosterone (KT), and (C) the gonadosomatic index (GSI). Data are mean (SEM) for three replicates at each of three fadrozole concentrations (μg/l) and the control. Asterisks (*) indicate significant difference from the control.
eggs/female/day. Reductions relative to controls in the 10 and 50 μg/l treatments were almost solely because the animals stopped spawning within 2 days of exposure. In the 2-μg/l treatment group, decreased fecundity was due to a slight (~10%) reduction in the number of spawns and about a 50% decrease in the number of eggs/spawn compared to controls (data not shown). Exposure to fadrozole did not significantly affect hatching success; mean (SEM) hatch in the control, 2, 10, and 50 μg/l treatments were 91.2 ± 2.7, 91.2 ± 3.9, 94.0 (n = 1), and 97.7% (n = 3), respectively.

FIG. 4. (A) Section of control ovary showing mature follicles typical of an actively spawning female. (B) Section of ovary from female exposed to fadrozole (50 μg/l) showing follicles undergoing atresia (e.g., at arrows) rather than proceeding to maturity. Scale bar = 295 μm.
DISCUSSION

A model CYP19 inhibitor, fadrozole, effectively inhibited brain aromatase activity, E2 biosynthesis, and vitellogenin production, as well as reproduction (fecundity) in a short-term assay with the fathead minnow. Brain aromatase activity in male and female fish exposed to a fadrozole concentration of about 50 μg/l was significantly decreased to about 18% of control values. Although we did not measure ovarian aromatase activity, which likely directly controls circulating E2 concentrations, inhibition of brain aromatase activity was entirely consistent with an observed reduction in plasma concentrations of E2 in female fathead minnows, to essentially non-detectable levels in the highest fadrozole treatment. Maintenance of vitellogenin concentrations in oviparous vertebrates, including fish, is achieved via activation of the estrogen receptor(s) by E2 (Specker and Sullivan, 1994). Results of the present study reflect this, in that a concentration-dependent decrease observed in plasma concentrations of vitellogenin, to near the limits of detection of the ELISA, closely resembled the concentration-response relationship for reductions in E2. Accompanying these responses were marked alterations in ovarian histology and a reduction in fecundity of the fish. This linkage of mechanism-specific information (aromatase inhibition) to a cascade of events through the endocrine system (reductions in E2) and target tissues (liver, ovary), to adverse effects in the whole organism (reduced fecundity) provides a novel demonstration of the behavior of aromatase inhibitors in female fish (as well as, quite probably, other oviparous vertebrates).

In males, exposure to fadrozole caused a significant increase in plasma concentrations of both T and KT. The cause of this is uncertain. It seems unlikely that inhibition of CYP19 would increase androgen concentrations in males by blocking conversion of T to E2, because (1) plasma E2 concentrations in males normally are small (Jensen et al., 2001), and (2) T concentrations in females from this study were not increased despite a clear inhibition of E2 synthesis. Based on this, we speculate that the fadrozole may have inhibited some aspect of androgen degradation/excretion specific to the males. An additional observation of interest in the males was the occurrence of a relatively unique histopathology in the gonads. Specifically, there was a notable, concentration-dependent enlargement of the seminiferous tubules accompanied by an abundant accumulation of sperm in their lumina. The marked accumulation of sperm in testes at the two highest fadrozole levels could be due to enhanced sperm production related to the increase in plasma T and KT (Afonso et al., 2000).

This study is the first with a small fish model to comprehensively assess the effects of a known aromatase inhibitor on reproductive fitness and associated endocrinology. Several of the responses observed were consistent with expectations based on studies with other classes of vertebrates (Elbrecht and Smith, 1992; Schieweck et al., 1988; Steele et al., 1987).
including limited experimentation with fish (Afonso et al., 1999, 2000; Kitano et al., 2000). Afonso et al. (1999) found that intraperitoneal injection of female coho salmon with fadrozole during late vitellogenesis (~1.5 months before spawning) significantly decreased plasma E2 concentrations, as well as ovulation (fecundity) of the fish. Injection of male salmon with fadrozole during this same period of sexual maturation advanced spermiation relative to controls; there also was some indication of increased concentrations of plasma T and KT in fish exposed to the aromatase inhibitor. Due to variations in route and timing of exposures, particularly in the context of the differential reproductive physiology of cyprinids versus salmonids, it is difficult to directly compare our results to those of Afonso et al. (1999, 2000). However, the effects of fadrozole on plasma steroid concentrations in male and female coho salmon and fathead minnows were qualitatively similar, as was the observation of reduced fecundity of females exposed to the aromatase inhibitor.

This study provides critical data relative to proposed EDC testing with the fathead minnow. First, it is clear that the suite of endpoints evaluated in the short-term reproduction assay described by Ankley et al. (2001) should effectively identify substances that inhibit aromatase activity, both as reproductive toxins and with respect to MOA. Fadrozole decreased fecundity of the fish, which was accompanied by marked alterations in gonadal histopathology in both sexes. These types of whole-animal and tissue-level alterations, while important to assessing the potential hazard of chemicals, do not directly implicate any particular toxic MOA. However, the profound reductions in circulating E2 were diagnostic of aromatase inhibition by fadrozole. This finding highlights the value of steroid determinations as a routine measurement in EDC screening/testing with fish. Because of the relatively small volumes of blood obtained from individual fathead minnows, many of the laboratories that utilize this species for EDC testing do not routinely collect steroid data. It is possible, however, through optimization of RIA techniques, to reliably measure E2, T, and KT in individual fathead minnows (Jensen et al., 2001; U.S. EPA, 2001). The ability to do so adds information not only as to the identification of MOA (as in the present study), but also is relevant to the interpretation of the potential risk of EDCs in the environment, where observations of decreased steroid concentrations in fish exposed to complex mixtures of contaminants, such as some types of effluents, are common (McMaster et al., 1996).

Decreases in E2 were accompanied by a reduction in plasma vitellogenin concentrations, a response consistent with expectations based on basic reproductive endocrinology of oviparous vertebrates (Specker and Sullivan, 1994). There has been an emphasis on alterations in vitellogenesis as indicative of EDC exposure in fish in general and the fathead minnow in particular. However, virtually all of this attention has concerned the physiologically abnormal induction of vitellogenin in male and/or juvenile fish exposed to estrogen receptor agonists (Ankley et al., 2001; Harries et al., 2000; Korte et al., 2000; Kramer et al., 1998; Nichols et al., 1999; Panter et al., 1998, 2002; Tyler et al., 1999). Results of the present study indicate that decreases in vitellogenin concentrations in females may be just as useful for identification of EDCs that inhibit estrogen synthesis and/or signaling as induction of the protein in males is for targeting estrogen receptor agonists. Panter et al. (2002) reported that the relatively potent estrogen receptor antagonist ZM 189,154 caused small decreases of plasma vitellogenin concentrations in mixed-sex juvenile fathead minnows. Failure of the antagonist to elicit a stronger response in that study might be related to the normally small concentrations of

![FIG. 6. Cumulative fecundity of fathead minnows prior to and after exposure to fadrozole for 21 days. Each line depicts the mean cumulative fecundity (assessed daily) of three replicates at each of three fadrozole concentrations (µg/l) and the control.](image_url)
vitellogenin (and, by inference, E2) in juvenile fish compared to that in sexually mature adult females. Based on the marked decrease in vitellogenin concentrations observed in the present study, we speculate that inhibition of vitellogenesis, either through alterations in steroid synthesis or antagonism at the level of the receptor, would be more pronounced in spawning females (actively sequestering vitellogenin in oocytes) than in juvenile fish or sexually dormant adults.

To date, most attention relative to the ecological effects of EDCs has been on compounds with the potential to act as estrogen mimics, in particular those that activate the estrogen receptor(s) (e.g., Desbrow et al., 1998; Folmar et al., 1996; Nichols et al., 1999; Purdom et al., 1994). Recently, there also has been some consideration of the occurrence and effects in fish of androgen receptor agonists in effluents associated with pulp and paper mills (Bortone et al., 1989; Jenkins et al., 2001; Larsson et al., 2000; Parks et al., 2001). Little is known, however, about chemicals in the environment that might exert adverse effects through alterations in enzymes involved in steroidogenesis, including CYP19. Alterations in endocrine function and reproduction in fish exposed to complex pulp and paper mill effluents from some locations are not inconsistent with effects on steroid metabolism (McMaster et al., 1996); however, specific chemicals and mechanisms associated with those observations have not been well defined. In a recent study, Noaksson et al. (2001) reported an association between decreased brain aromatase activity, circulating E2 concentrations, and GSI in female perch from a contaminated lake in Sweden. The results of our study with fadrozole clearly demonstrate that inhibitors of CYP19 in fish can result in significant adverse effects on reproductive fitness. This observation, coupled with more indirect evidence of inhibition of steroidogenesis in wild fish populations (e.g., McMaster et al., 1996; Noaksson et al., 2001), indicates the need for further consideration of aromatase inhibition as an MOA in assessing the ecological consequences of EDCs.

Controlled experimentation with single chemicals to identify causal (or correlative) relationships across different biological levels of organization can be critical to diagnostic assessments of adverse effects in wild fish populations. Three of the endpoints evaluated in this study, plasma steroid and vitellogenin concentrations and aromatase activity, have been used to varying degrees as “biomarkers” in studies focused on assessments of the possible effects of EDCs in fish from the field (e.g., McMaster et al., 1996; Noaksson et al., 2001; Sumpter and Jobling, 1995). There is little doubt that these types of endpoints can effectively indicate whether an animal had been exposed to a chemical(s) with a particular MOA, but these (or other) biomarkers are often questioned as to the interpretation of their biological significance in terms of fitness of the animal (Ankley et al., 1997). Usually, it is a question of whether or not adverse effects are occurring in individuals (and populations) that is ultimately of concern to risk assessors/resource managers. For example, from an EDC perspective, there has been considerable debate as to whether observations of vitellogenin induction in male fish from the field can be correlated with adverse reproductive outcomes, or whether this response is only an indication of exposure to estrogen receptor agonists (Ankley et al., 1997). Although the present study does not lend insight to that question, our data do indicate that, in reproducively mature female fathead minnows, decreases in (brain) aromatase activity can be associated with reduced plasma E2 and, subsequently, with decreases in vitellogenin concentrations. Further, these alterations are directly correlated, perhaps causally, with decreased fecundity of the fish. Hence, for these biomarkers (at least in the context of actively spawning females), there appears to be a reasonable basis for their utility, not only in identifying exposures to chemicals with a given MOA, but also in prediction of adverse effects at the level of the whole organism and, perhaps, through linkage to appropriate models, populations.

ACKNOWLEDGMENTS

We thank Drs. Heiko Schoenfuss and Sigmund Degitz for review comments on an earlier version of the manuscript. Diane Spehar and Roger LePage assisted in manuscript preparation.

REFERENCES


EFFECTS OF AN AROMATASE INHIBITOR ON FISH


