Effects of Atrazine on Sexual Maturation in Female Japanese Quail Induced by Photostimulation or Exogenous Gonadotropin

Kelly W. Wilhelms,†‡ Sara A. Cutler,‡ John A. Proudman,§ Lloyd L. Anderson,‡ and Colin G. Scanes*†‡

†Interdepartmental Toxicology Program, ‡Department of Animal Science, Iowa State University, Ames, Iowa 50011, USA
§U.S. Department of Agriculture-Agricultural Research Service, Biotechnology and Germplasm Laboratory, Beltsville, Maryland 20705

(Received 17 January 2005; Accepted 8 July 2005)

Abstract—The herbicide atrazine has gained recent attention for its reported effects on reproduction in amphibians. The present study examined the putative effects of atrazine during sexual maturation in the photostimulated female Japanese quail. Furthermore, the effects of atrazine on birds administered exogenous gonadotropin (pregnant mare serum gonadotropin [PMSG]) were investigated. Atrazine was administered up to 1,000 ppm in the diet to female quail undergoing photoperiodically induced sexual maturation. At high dietary concentrations, atrazine exhibits signs of overt toxicity with reductions in growth, feed intake, and liver weights, but these effects were dependent on the timing of treatment administration. Atrazine did not influence the weights of reproductive tissues (ovary and oviduct) or circulating concentrations of luteinizing hormone (LH). However, high concentrations of atrazine depressed circulating concentrations of estradiol. Treatment with atrazine for four weeks during sexual maturation inhibited growth but did not affect any other parameter assessed (feed intake, liver, ovary, or oviduct weights or the circulating concentrations of LH and estradiol). In birds receiving daily injections of PMSG, atrazine reduced growth, feed intake, and liver weights. However, PMSG-induced gonadal and oviduct growth was not affected by atrazine. The present results suggest that dietary atrazine exhibits limited reproductive toxicity in female quail during sexual maturation and only at concentrations above ecological relevance.

Keywords—Atrazine, Female quail, Photostimulation, Sexual maturation, Pregnant mare serum gonadotropin

Introduction

The herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a widely used pesticide in the United States, with usage up to 36,000 metric tons per year ([1]; http://www.epa.gov/oppbead1/pestsales/01pestsales/market_estimates2001.pdf). It is persistent in the environment (up to one year under ideal conditions) and is relatively water soluble (28 mg/L at 20°C) [2]. Recently, atrazine has come under scrutiny as a putative endocrine disruptor.

Concentrations of atrazine as low as 0.1 ppb have been reported to feminize male amphibians [3,4]. This effect does not appear to be due to the direct activation of the estrogen receptor (ER), as atrazine does not bind to either the rat or the human ER in vitro [4–8]; conversely, atrazine may be a weak antiestrogen [6,9]. Alternatively, it has been hypothesized that the feminizing effect of atrazine is due to activation of aromatase [3], as has been demonstrated in cultured adrenocortical carcinoma cells [10].

At much higher concentrations, atrazine exhibits reproductive toxicity in female rats. For example, high doses of atrazine (administered by oral gavage) decrease circulating concentrations of both luteinizing hormone (LH) [11] and prolactin [11,12]. Furthermore, atrazine [13,14] and its metabolites [15] delay the onset of puberty in rats. Moreover, atrazine disrupts the estrous cycle and initiates a premature reproductive senescence [11,16–18]. Atrazine blocks embryo implantation and reduces plasma concentrations of progesterone in some rat lines [19]. However, little effect exists on plasma concentrations of estradiol (limited to the Sprague-Dawley rat) and follicle-stimulating hormone (FSH) [11,16,19]. Similarly, in the adult female pig, treatment with dietary atrazine inhibits the progression of the estrous cycle and suppresses gonadotropin-releasing hormone–induced secretion of LH [20,21].

Birds may be exposed to atrazine in the temperate zone, particularly during the spring application period. Atrazine exhibits low acute toxicity in birds with a dietary lethal concentration killing 50% (LC50) greater than 5,000 ppm [22]. However, to date, little information exists on the effect of atrazine on reproduction in birds. Reproductive studies submitted to the U.S. Environmental Protection Agency describe a dietary no-observed-adverse-effect concentration of 225 ppm and a lowest-observed-adverse-effect concentration of 675 ppm ([23]; http://www.epa.gov/oppsrrd1/reregistration/atrazine). At the lowest-observed-adverse-effect concentration, reduced egg production, an increase in defective eggs, and reduced embryo viability are observed in the northern bobwhite quail and mallard duck [23].

The present studies examine the effects of atrazine on reproductive development in female Japanese quail, sexual maturation being induced by increasing the day length from 8:16 h light:dark to 16:8 h light:dark. Under these circumstances, ovarian and oviductal tissues are undergoing rapid logarithmic growth because of the photoperiodic stimulation of the hypothalamo–pituitary–gonadal axis [24]. It has been reported that growth of the oviduct is a sensitive endpoint for estrogenicity in birds [25]. Similarly, the developing oviduct is sensitive to antiestrogenic compounds (e.g., [26]). As such, growth of the reproductive tissues and circulating concentrations of estradiol and LH were used as endpoints for adverse effects of atrazine on reproduction (e.g., antiestrogenic or interference with the action of gonadotropin).
Experimental design and treatments

The effects of atrazine on photoinduced sexual maturation were studied in four separate experiments using dietary administration. Atrazine (CAS 1912-24-9, 99.9% purity; Chem Service, West Chester, PA, USA) or tamoxifen (a positive control for antiestrogenicity) (Sigma-Aldrich, St. Louis, MO, USA) was weighed and directly added to Purina Game Bird Chow to obtain the concentrations as outlined in each of the studies. These diets were mixed for 20 min and stored at room temperature for the duration of each study. In preliminary work, concentrations of atrazine were measured in the feed using gas chromatography. These results, coupled with the consistent response of the birds to the positive control (small volumes of tamoxifen added to the diet), suggested that this mixing protocol was suitable to distribute the compounds uniformly throughout the feed. The concentrations of atrazine and tamoxifen are considered nominal.

A summary addressing the goals of each study is presented in Table 1. Studies 1 and 2 examined the effect of atrazine on sexual maturation in the photostimulated female quail. Previous reports have suggested that atrazine exhibits a weak antiestrogenic effect [6,9]. As such, it was hypothesized that atrazine would exert an antiestrogenic effect on sexual maturation in female quail. In study 1, four-week-old quail were weighed and randomly assigned to cages, three per cage, three cages per treatment (nine birds per treatment). The birds received the various treatments (negative control [0 ppm atrazine], atrazine, or the antiestrogen tamoxifen) in the diet and had water available ad libitum for two weeks under long photoperiods (16:8 h light:dark). The dietary concentrations of atrazine were 0, 1, 10, 100, and 1,000 ppm. As a positive control, a group of quail received treatment with 100 ppm tamoxifen in the diet. In experiment 2, four-week-old birds were photostimulated for two weeks before treatment. At six weeks of age, the birds were weighed and randomly assigned to cages, two per cage, five cages per treatment (10 birds per treatment). This design was dictated by the availability of birds, and thus a positive control was not run. The birds were assigned to the following treatment groups: control and 1,000 ppm atrazine. Treatment diets and water were available ad libitum under long photoperiods (16:8 h light:dark) for two weeks (total duration of photostimulation: four weeks).

Experiment 3 examined the effect of longer exposure to atrazine (four weeks) during sexual maturation and the reversibility of the toxic effects of atrazine observed in the first two weeks of exposure. Four-week-old birds were weighed and randomly assigned to cages, three per cage, three cages per treatment (nine birds per treatment). The birds received dietary treatments of 0 or 1,000 ppm atrazine for two or four weeks. Feed and water were available ad libitum. The recovery group was fed 1,000 ppm atrazine for two weeks and then control feed for the following two weeks. All treatments were administered under long photoperiods (16:8 h light:dark).

In experiment 4, the effects of atrazine on gonadotropin-induced sexual maturation were examined. It was hypothesized that atrazine would interfere with the effects of gonadotropin on ovarian development. The gonadotropic effects of pregnant-mare serum gonadotropin (PMSG) on female reproduction in the fowl have been well characterized (e.g., [27]). Furthermore, this hormone is relatively inexpensive and readily available from commercial suppliers. As such, PMSG (Sigma-Aldrich) was used to simulate increased secretion of FSH in the female quail [27]. Four-week-old birds were weighed and randomly assigned to cages, three per cage, three cages per treatment (18 birds per treatment). They were administered dietary treatments of 0 or 1,000 ppm atrazine and water ad libitum for two weeks under long photoperiods. These groups were further subdivided into treatments of daily subcutaneous injections of saline (vehicle control) or 10 IU/d PMSG (delivered in 0.1 ml saline) (Sigma-Aldrich). Injections were administered between 8:00 AM and 10:00 AM each day.

At the end of each treatment period, the quail were weighed and killed by decapitation, blood samples were taken, and the tissues were dissected and weighed (oviducts were weighed dry). Blood plasma samples were stored at −20°C before hormonal analysis.

Hormone analysis

Plasma concentrations of estradiol (E2) were determined using a commercial enzyme-linked immunosorbent assay system (DRG-International, Mountainside, NJ, USA). This assay has been used by other researchers to measure circulating concentrations of estradiol in the Japanese quail [28]. As this system is designed for human serum or plasma, validation was
Atrazine and sexual maturation in female quail

Fig. 1. Effect of atrazine on indices of general toxicity in sexually maturing female Japanese quail. Different from control, *p < 0.05, **p < 0.01, ***p < 0.001. (A) Growth (average daily gain); (B) feed intake; (C) liver weight; (D) liver-somatic index.

RESULTS

Effect of atrazine on sexual maturation in female Japanese quail

No deaths occurred in any treatment group in any of the studies, and thus atrazine had no effect on mortality in female quail during sexual maturation. In contrast, treatment with atrazine in the diet during the first two weeks of sexual maturation (on a stimulatory photoperiod) exerted general toxicity in the female quail. For example, atrazine at 1,000 ppm decreased (p < 0.05) growth (average daily gain) and feed intake by 14.3 and 11.1%, respectively (Fig. 1A and B). Furthermore, atrazine decreased (p < 0.05) liver weight by 18.8% versus control (Fig. 1C). With atrazine decreasing body weight, the liver was proportionately smaller with no effects of atrazine on the liver index (liver as a percentage of body wt) (Fig. 1D). The antiestrogen tamoxifen reduced (p < 0.05) feed intake and liver weight by 11.1 and 23.4%, respectively, versus control (Fig. 1B and C). In addition, tamoxifen tended to reduce (p < 0.10) growth (Fig. 1A).

The ability of atrazine to block gonadal development during sexual maturation in female quail was examined. It was hypothesized that atrazine acts as an antiestrogen. However, atrazine had no effect on weights of the ovary or oviduct (Fig. 2A–D). In addition, atrazine did not influence circulating concentrations of LH (Fig. 3A). However, circulating concentrations of estradiol were reduced 80.8% compared to control (p < 0.05) (Fig. 3B). In contrast, tamoxifen decreased (p < 0.05)
Fig. 2. Effect of atrazine on indices of reproductive development in sexually maturing female Japanese quail. Different from control, * $p < 0.05$, *** $p < 0.001$. (A) Ovary weight; (B) ovary-somatic index; (C) oviduct weight; (D) oviduct-somatic index.

Fig. 3. Effect of atrazine and interactions between atrazine and photoperiod on circulating concentration of luteinizing hormone (LH) and estradiol in sexually maturing female Japanese quail. Different from control, * $p < 0.05$. Birds represented in (A) and (B) were initially photostimulated on day 0 of treatment. Birds represented in (C) and (D) were initially photostimulated two weeks before treatment. (A) Circulating luteinizing hormone; (B) circulating estradiol; (C) circulating luteinizing hormone; (D) circulating estradiol.
with exposure to atrazine (4A). Circulating concentrations of estradiol tended to decrease weights (Table 3) or circulating concentrations of LH (Fig. 4A). Atrazine had no effect on feed intake, liver, ovary, or oviduct weights (Table 2) or on circulating concentrations of LH and estradiol were observed (Fig. 3C and D). However, no other indices were different from the control or atrazine group (two weeks atrazine followed by two weeks of recovery) (Table 3). The studies described are among the first to characterize the effects of atrazine on sexual maturation in birds [30]. With an avian dietary LC50 greater than 5,000 ppm [22], atrazine would be expected to exhibit low acute toxicity in quail. During the course of these studies, no mortality was observed, consistent with the high LC50. Atrazine suppressed growth in birds receiving treatment for up to four weeks (Fig. 1A and Table 3). However, feed intake and liver weights were inconsistent with gonadotropic actions on the ovary. The results of this study are summarized in Table 4. No detrimental effects of atrazine alone or in the presence of PMSG were observed on reproductive development compared with controls. Atrazine treatment alone for two weeks depressed (p < 0.05) growth by 24.0% versus the respective control. However, no effects of atrazine alone were observed on feed intake, liver, ovary, or oviduct weights (Table 4) or on plasma concentrations of LH (data not shown). As might be expected, subcutaneous injections of PMSG (10 IU/d) increased (p < 0.05) liver weights by 32.2% versus saline alone. In addition, ovary and oviduct weights were increased 1.8- and 26-fold in quail receiving PMSG injections and no atrazine in the diet versus the saline controls. Atrazine in the diet along with the daily injections of PMSG decreased (p < 0.05) growth and feed intake 23.1 and 15.0%, respectively, versus quail receiving PMSG alone. Moreover, atrazine reduced (p < 0.05) liver weights 28.2% in PMSG-treated birds. However, no effects of atrazine were observed on PMSG-induced growth of the ovary or oviduct (Table 4) or on plasma concentrations of LH (data not shown).

**DISCUSSION**

The ability of atrazine to block gonadotropin-induced sexual maturation in the female quail was examined in experiment 4. It was hypothesized that atrazine would directly interfere with the reversibility of any toxicity of atrazine observed. The results are shown in Table 3. Atrazine (1,000 ppm in the diet) decreased growth (p < 0.01) 23.8% versus control. However, atrazine had no effect on feed intake, liver, ovary, or oviduct weights (Table 3) or circulating concentrations of LH (Fig. 4A). Circulating concentrations of estradiol tended to decrease with exposure to atrazine (p = 0.09) (Fig. 4B). In the recovery group (two weeks atrazine followed by two weeks of recovery), growth was depressed 14.3% versus control (Table 3) but was not different from the atrazine group (p > 0.05). However, no other indices were different from the control or atrazine groups (feed intake, liver, ovary, and oviduct weights [Table 3] and plasma concentrations of LH and estradiol [Fig. 4A and B]).

**Effects of atrazine on reproductive development induced by exogenous gonadotropin**

The ability of atrazine to block gonadotropin-induced sexual maturation in the female quail was examined in experiment 4. It was hypothesized that atrazine would directly interfere with gonadotropic actions on the ovary. The results of this study are summarized in Table 4. No detrimental effects of atrazine alone or in the presence of PMSG were observed on reproductive development compared with controls. Atrazine treatment alone for two weeks depressed (p < 0.05) growth by 24.0% versus the respective control. However, no effects of atrazine alone were observed on feed intake, liver, ovary, or oviduct weights (Table 4) or on plasma concentrations of LH (data not shown). As might be expected, subcutaneous injections of PMSG (10 IU/d) increased (p < 0.05) liver weights by 32.2% versus saline alone. In addition, ovary and oviduct weights were increased 1.8- and 26-fold in quail receiving PMSG injections and no atrazine in the diet versus the saline controls. Atrazine in the diet along with the daily injections of PMSG decreased (p < 0.05) growth and feed intake 23.1 and 15.0%, respectively, versus quail receiving PMSG alone. Moreover, atrazine reduced (p < 0.05) liver weights 28.2% in PMSG-treated birds. However, no effects of atrazine were observed on PMSG-induced growth of the ovary or oviduct (Table 4) or on plasma concentrations of LH (data not shown).

**Table 2. Effects of atrazine on indices of growth and reproduction during sexual maturation in four-week-old female Japanese quail. Birds were initially photostimulated two weeks prior to administration of atrazine.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average daily gain (g)</th>
<th>Daily feed (g)</th>
<th>Liver (g)</th>
<th>Liver index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.4 ± 0.43</td>
<td>21 ± 1.0</td>
<td>3.7 ± 0.25</td>
<td>2.3 ± 0.16</td>
</tr>
<tr>
<td>1,000 ppm atrazine</td>
<td>2.4 ± 0.44</td>
<td>20 ± 1.1</td>
<td>4.1 ± 0.58</td>
<td>2.6 ± 0.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ovary (mg)</th>
<th>Ovary index (%)</th>
<th>Oviduct (mg)</th>
<th>Oviduct index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,769 ± 641</td>
<td>1.1 ± 0.39</td>
<td>2,834 ± 604</td>
<td>1.8 ± 0.37</td>
</tr>
<tr>
<td>1,000 ppm atrazine</td>
<td>1,764 ± 755</td>
<td>1.1 ± 0.49</td>
<td>2,459 ± 688</td>
<td>1.6 ± 0.45</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01 from control.
Effects of dietary atrazine (1,000 ppm) on exogenous gonadotropin (PMSG) stimulated growth and sexual development in female tradiol.

Unlike letters indicate difference (*p = 0.09). (A) Circulating luteinizing hormone; (B) circulating estradiol.

Consistently affected by treatment with atrazine. Furthermore, birds that were photostimulated before administration of atrazine exhibited no overt toxicity (Table 2).

Treatment with high concentrations of atrazine in the diet under long photoperiods and/or with daily injections of PMSG depressed growth, feed intake, and liver weights (Table 4). The lack of changes in the liver-somatic index (relative wt of liver compared to body wt; Fig. 1D and Table 4) may suggest that the changes observed resulted from feed restriction/aversion, not atrazine toxicity. Similarities between atrazine treatment and feed restriction have been demonstrated in male quail during sexual maturation [30]. In addition, the effects of atrazine administered concurrently with the initiation of long day lengths (studies 1 and 3) are not consistent with quail photostimulated before receiving atrazine in the diet (study 2). It is speculated that the timing of treatment influences these parameters, perhaps because of the physiological/hormonal/metabolic changes occurring with photostimulation and/or sexual maturation. These effects on growth, feed intake, and liver weights were of a greater magnitude when sexual maturation was being rapidly induced by increased photoperiod coupled with PMSG injections (Table 4). During sexual maturation, demands on physiological and metabolic systems increase, including those that mediate growth/maturation of the reproductive organs, yolk formation, and calcium metabolism [31]. Disruption of nutrient availability through reduced feed intake may explain the effects on growth observed in atrazine-treated birds, perhaps illustrating the paradigm that the physiological state of the animal influences its sensitivity to direct or indirect effects of a toxicant. The increase in sensitivity to atrazine observed in the present studies suggests that excessive exposure to a toxicant, perhaps including atrazine, may influence growth of the bird at a time where quail and other birds are undergoing sexual maturation in the spring.

In birds, long photoperiods markedly increase pulsatile release of the pituitary gonadotropins FSH and LH [31]. As in mammals, in birds, stimulation of the ovary by gonadotropins increases production of estradiol and other reproductive hormones [32]. Estradiol, in turn, stimulates rapid growth and secretory activity of the oviduct [33]. As is to be expected, increases in ovarian weights were observed with photostimulation, ovarian weights increasing to 137 mg and greater than 1,500 mg, respectively, after two and four weeks on long daily photoperiods (Fig. 2A and Table 3). Similarly, massive growth of the oviduct occurred, oviduct weights in short-day quail being 20 mg [34,35] compared to 40 and 2,500 mg, respectively, after two and four weeks on long daily photoperiods (Fig. 2C and Table 3). Atrazine [13,14] and its metabolites [15] have been reported to delay the onset of puberty in rats. In contrast, no overt effects of atrazine at approximately 128.5 mg/kg/d (calculated from 1,000 ppm) were observed on sexual maturation of the gonad or oviduct in the female quail. It is speculated that the timing of treatment influences these effects of a toxicant. The increase in sensitivity to atrazine observed in the present studies suggests that excessive exposure to a toxicant, perhaps including atrazine, may influence growth of the bird at a time where quail and other birds are undergoing sexual maturation in the spring.

Table 4. Effects of dietary atrazine (1,000 ppm) on exogenous gonadotropin (PMSG) stimulated growth and sexual development in female Japanese quail

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Indices of general toxicity</th>
<th>Indices of reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average daily gain (g)</td>
<td>Daily feed (g)</td>
</tr>
<tr>
<td>Saline</td>
<td>2.5 ± 0.06A</td>
<td>18 ± 0.4A</td>
</tr>
<tr>
<td>Atrazine</td>
<td>1.9 ± 0.19B</td>
<td>17 ± 0.2A</td>
</tr>
<tr>
<td>Pregnant-mare serum gonadotropin</td>
<td>2.6 ± 0.20A</td>
<td>20 ± 0.7B</td>
</tr>
<tr>
<td>Pregnant-mare serum gonadotropin-atriazine</td>
<td>2.0 ± 0.14B</td>
<td>17 ± 0.3A</td>
</tr>
</tbody>
</table>

Unlike letters indicate difference (*p < 0.05).
of sexual maturation may have masked reproductive toxicity of atrazine as observed on the weights of the reproductive tissues (e.g., Table 3).

In female rats, high doses of atrazine (administered by oral gavage) decrease circulating concentrations of LH [11]. In contrast, in the present study, no effects of atrazine were observed on plasma concentrations of LH—even at very high concentrations in the diet (Figs. 3A and C and 4A). Moreover, in the bird, circulating concentrations of estradiol were reduced by atrazine (Fig. 3B) without changes in oviduct weight. While not conclusive, this effect may be reversible (recovery group; Fig. 4B). This is in contrast to the increase in serum estradiol seen in some rats [19] and to the hypothesized mechanism of aromatase induction in amphibians [3]. It is unclear why these changes in circulating concentrations of estradiol are not reflected by the size of the oviduct. However, this effect may be explained by diurnal variation in circulating concentrations of reproductive hormones (e.g., Gulati et al. [36]) or by oviduct weight being not as sensitive to fluctuations in plasma concentrations of estradiol as would be perceived.

In rats, atrazine has been reported to disrupt the estrous cycle, initiate a premature reproductive senescence [11,16–18], and reduce plasma concentrations of progesterone [16,19]. In the pig, atrazine inhibits the estrous cycle [20] and inhibits the gonadotropin-releasing hormone-mediated release of LH [21]. It is possible that the effects of atrazine observed in mammals are due either to an antiestrogen effect or to atrazine impairing the cellular response(s) to gonadotropins. In the present study, atrazine did not exhibit overt antiestrogenic activity, as oviduct weights were not different from control (e.g., Fig. 2C). Furthermore, atrazine did not influence ovarian development (as suggested by wt) or function (as suggested by oviduct development presumably due to estrogen production) when stimulated by exogenously administered gonadotropin (PMSG) (Table 4). These results may suggest that atrazine does not influence the cellular responses of the reproductive organs to gonadotropins.

The present studies employed high concentrations of atrazine to identify the putative reproductive toxicity of atrazine in the sexually maturing female quail. The results herein reveal that atrazine at approximately 128 mg/kg/d exhibits limited reproductive toxicity in the female bird during sexual maturation. These studies provide no evidence that atrazine affects hypothalamic control of sexual maturation or gonadotropic action on reproductive organ growth. Moreover, the concentration of atrazine observed to exert an effect on growth and circulating concentrations of estradiol is well above expected environmental concentrations. For example, concentrations of atrazine have been reported as high as 53 ppm in Australasian streams [37]. Furthermore, soil concentrations have been reported as high as 49 ppm [37]. In contrast, effects on the female quail were not observed at a dietary dose below 1,000 ppm. Based on the presented evidence, it is unlikely that atrazine at environmentally relevant concentrations would have a profound impact on avian populations.

Acknowledgement—This work was funded by a special U.S. Department of Agriculture grant through the Center for Designing Foods to Improve Nutrition, Iowa State University (Ames, IA, USA). We thank David F. Cox (Department of Statistics, Iowa State University) for statistical advice. We thank Jason Belden (Coats Laboratory, Department of Entomology, Iowa State University) for residue analysis of atrazine in preliminary studies.

REFERENCES

Environ. Toxicol. Chem. 25, 2006 239


