Atrazine Interaction with Estrogen Expression Systems

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1 Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is a herbicide used to control growth of many broadleaf weeds and some annual grasses, particularly in corn and sorghum crops (Vencill 2002). Atrazine inhibits plant photosynthesis by binding to the D1 protein of the protein-bound plastoquinone (Q_b) located in the plant thylakoid membrane (Devine et al. 1993), thereby preventing electron transfer at the reducing site of chloroplast complex II (Good 1961). Atrazine and its mono- and didealkylated metabolites are moderately mobile in soil (Qiao et al. 1996), are persistent in the environment, with a soil half-life of 17–26 d (Winkelmann and Kliane 1990), and are detected in surface water (Schottler et al. 1998). Because of these characteristics, the EPA Office of Drinking Water has established an annual average maximum contaminant level (MCL) for atrazine of 3 ppb (US EPA 1996). More recently, the Office of Pesticide Planning has determined that a 90-d average concentration of 12.5 ppb in finished drinking water is safe, based on the chronic no observable effect level (NOEL) of 1.8 mg/kg/d observed in a chronic rodent feeding study (US EPA 2006).

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2 Effects on the Hypothalamic-Pituitary-Gonadal (HPG) Axis in Rodents

Acute or chronic treatment of female Sprague–Dawley (SD) or Long Evans (LE) rats with atrazine disrupts estrous cycling (Eldridge et al. 1994, 1999a,b; Simpkins et al. 1998; Cooper et al. 2000). Mode of action research has established that this disruption may be attributed to atrazine inhibition of a surge of pituitary luteinizing hormone (LH) necessary for rodent ovulation (Simpkins et al. 1998; Eldridge et al. 1999a; Cooper et al. 2000). Long-term feeding studies of intact SD female rats with atrazine also demonstrated a significantly increased incidence, or earlier lifetime appearance of mammary tumors; such tumors were attributed to prolonged exposure to endogenous estrogen in atrazine-treated animals that developed a state of constant estrus earlier in life (neuroendocrine aging) than did the controls (Eldridge et al. 1994, 1999a; Stevens et al. 1999; Wetzel et al. 1994). The U.S. Environmental Protection Agency (US EPA 2002) and other regulators (IARC 1999; European Union 2000; United Kingdom Pesticide Directorate 2000; APVMA 2004) concluded that the mammary tumor response observed in female SD rats was not relevant to humans because of differences between species in the mechanisms of neuroendocrine aging of the HPG axis.

Because ovarian estrogens mediate neuroendocrine control of pituitary gonadotropin surges, the question arose as to whether the mechanism of atrazine effects on the estrous cycle in female rats might include direct interaction with estrogen expression.

Indeed, some authors suggest that atrazine is "estrogenic" or is an "environmental estrogen" (Davis et al. 1993; Steingraber 1997; Muir et al. 2004; Tanaka et al. 2004), despite the absence of supporting evidence in the literature. Environmental estrogens have long been suspected of etiological involvement in hormone-dependent cancer (Sonnenschein and Soto 1998; Mukherjee et al. 2006), although firm association has been difficult to establish (Calle et al. 2002; Safe 2004; Mitra et al. 2004). The hypothesis that atrazine is estrogenic has been repeatedly evaluated in the published literature over the years. Several dozen studies have addressed whether atrazine can imitate, inhibit, or otherwise modulate estrogen expression, in a wide variety of animal and *in vitro* models. This review addresses and summarizes the results of these studies.

3 Studies on Estrogen Expression Systems

3.1 Estrogen-Mediated Expression In Vivo

Table 1 presents an overview, taken from the literature, of responses to atrazine exposure during testing of estrogen expression *in vivo*.

Table 1 Responses to atrazine exposure using *in vivo* tests of estrogen expression

Species	Tissue	Stimulated[a]	Inhibited[a,b,c]	Reference
Rat, OVX	Mammary tumor promotion	No	—	Stevens et al. 1999
Rat, OVX	Uterine weight	No	Weak	Tennant et al. 1994a
Rat, OVX	Uterine weight	No	Weak	Connor et al. 1996
Rat, OVX	Progesterone receptor expression	No	Weak	Tennant et al. 1994a
Rat, OVX	Progesterone receptor expression	No	Weak	Connor et al. 1996
Rat, OVX	Progesterone receptor mRNA	No	No	McMullin et al. 2004
Rat, OVX	Uterine thymidine incorporation	No	Weak	Tennant et al. 1994a
Rat, OVX	Uterine peroxidase reaction	No	Weak	Connor et al. 1996
Rat, OVX	Transplanted pituitary tumor	No	—	Fujimoto and Honda 2003
Rat, intact	Reproductive tract development	No	—	Eldridge et al. 1998
Rat, intact	Vaginal cytology cornification	No	—	Eldridge et al. 1999b
Rat, intact	Prepubertal uterine weight	No	—	Ashby et al. 2002
Rat, intact	DMBA-induced tumor growth	No	Inconclusive	Tanaka et al. 2004
Rat, OVX	Estrogen-primed LH surge	—	Weak	Cooper et al. 2000
Rat, OVX	Estrogen-primed LH surge	—	Weak	Eldridge et al. 1999a
Rat, OVX	Estrogen-primed LH surge	—	Weak	Simpkins et al. 1998
Rat, OVX	Estrogen-primed LH surge	—	Weak	McMullin et al. 2004
Alligator	Eggs, female phenotype	No	—	Crain et al. 1999
Crocodile	Eggs, female phenotype	No	—	Beldomenico et al. 2007
Turtle	Feminization of testicular tissue	No	—	De Solla et al. 2006
Frog	Female sex ratio of tadpoles	No	—	Carr et al. 2003
Goldfish	Vitellogenin production	No	—	Spano et al. 2004
Carp	Vitellogenin production	No	No	Sanderson et al. 2001
Zebrafish	Vitellogenin mRNA	No	—	Muncke et al. 2007
Minnow	Various female reproductive parameters	No	—	Bringolf et al. 2004
Japanese quail	Female oviduct weight, serum LH	No	—	Wilhelms et al. 2006
Japanese quail	Feminization of male reproductive tract	No	No	Wilhelms et al. 2005
Fruit flies	Yolk protein genes	No	No	LeGoff et al. 2006

OVX, ovariectomized; —, assessment not done; DMBA, dimethylbenzanthracene; LH, luteinizing hormone.

[a] No, indicates no response to atrazine was observed at doses that were at least 10^5 molar excess of the effective dose of estradiol.

[b] Weak, indicates a response to atrazine was observed only at doses that were at least 10^5 in excess of the effective dose of estradiol.

[c] Inconclusive, no dose response or no historical control data for DMBA-treated rats.

A reproductive toxicology study in rats fed atrazine in the diet at concentrations up to 500 ppm gave early evidence that *in vivo* exposure to atrazine was not estrogenic (Hauswirth and Wetzel 1998). Estrogen-dependent responses such as feminization of immature males, premature puberty in females, and infertility were not observed in this study. Additional insights were also obtained from chronic feeding studies. Although several chronic feeding studies with atrazine in intact SD female rats had yielded an increased or earlier lifetime incidence of mammary tumors, the tumor response failed to appear in studies with ovariectomized (OVX) female rats (Stevens et al. 1999). In atrazine-treated OVX rats, well-established estrogen target organs such as mammary and uterine tissues remained rudimentary and appeared unstimulated after long-term feeding at doses that exceeded the Maximum Tolerated Dose (MTD) (Stevens et al. 1999).

Tennant and coworkers (1994a) published several studies demonstrating that atrazine, at acute MTD oral doses, failed to stimulate uterine weight of OVX rats. Incorporation of [^{3}H]thymidine into uterine DNA, a more specific index of estrogen-mediated promotion of tissue growth, was similarly not stimulated by atrazine, nor was expression of the uterine progesterone receptor, known to be highly specific for, and sensitive to, estrogen. Studies by Connor et al. (1996) confirmed that atrazine did not induce uterine weight or progesterone receptor responses; they added a test of uterine peroxidase expression, which atrazine also failed to stimulate. Similarly, McMullin et al. (2004) reported that the uterine progesterone receptor mRNA was not stimulated by atrazine in OVX rats. Another established estrogen-mediated response in rats, the release of prolactin from pituitary tissue transplanted under the animal's kidney capsule, was examined by Fujimoto and Honda (2003); results showed no evidence that atrazine stimulated prolactin release.

Detailed histological examination was conducted on vaginal, uterine, and mammary tissues from intact SD female rats administered atrazine; no treatment-related estrogenic responses were observed (Eldridge et al. 1998). Eldridge et al. (1999b) provided a detailed analysis of vaginal cytology and estrous cycling patterns in intact SD rats administered atrazine. Within the first few weeks of dosing, vaginal cytology appeared less cornified (i.e., diminished estrogenic response), suggesting, once again, that atrazine was not acting as an estrogen agonist. Ashby et al. (2002) administered atrazine to prepubertal female rats and did not observe estrogen-dependent responses, i.e., stimulation of uterine weight. Tanaka et al. (2004) administered dimethylbenzanthracene (DMBA) with and without atrazine to stimulate mammary tumor growth in intact young female rats. Administration of DMBA initiated and/or promoted tumor growth. The incidence of ovarian and mammary tumors was not increased in the DMBA + atrazine treatment groups. In fact, a non-dose-responsive decrease of ovarian tumors was observed in all atrazine-treated groups. This study bears replication because no historical control data were provided, which prevents knowing, with certainty, if the control incidence of ovarian tumors in DMBA-treated control rats was elevated above the normal range.

A number of studies have also been conducted with atrazine in nonmammalian species. Crain et al. (1999) reported a lack of feminization of alligator eggs, or stimulation of the immature reproductive tract after atrazine exposure; both are very

sensitive markers for estrogenic effects in reptiles. Similar results were recently reported for crocodile egg exposure by Beldomenico et al. (2007). De Solla et al. (2006) found that testicular development of snapping turtles was unaffected by incubation of eggs in soil containing atrazine. Although Hayes et al. (2002) reported that atrazine demasculinizes or feminizes developing male *Xenopus laevis* tadpoles (Hayes 2005; Hayes et al. 2006), Carr et al. (2003) did not find any effect of atrazine on the sex ratio of developing *Xenopus*. The result reported by Carr et al. (2003) was confirmed in a large study (Kloas et al. 2007) conducted concurrently in two laboratories. In this study, *Xenopus laevis* was exposed to atrazine at concentrations of 0.01, 0.1, 1.0, 25, or 100 ppb from day 8 postfertilization until the completion of metamorphosis; estradiol, administered under similar conditions, at a concentration of 0.2 ppb resulted in a significant increase in larvae with female or mixed sex gonads, compared to untreated controls. Bringolf et al. (2004) reported that atrazine had no effect on a number of reproductive tract structures in minnows.

Production of fish vitellogenin, a well-established estrogen-mediated response, has been examined after atrazine exposure in goldfish (Spano et al. 2004) and carp (Sanderson et al. 2001); no effect of atrazine treatment was noted in either study. Atrazine also failed to stimulate vitellogenin mRNA production in incubations of zebrafish embryos (Muncke et al. 2007). Wilhelms et al. (2006) did not find any evidence that atrazine affected uterine weight or pituitary LH release in quail. Earlier, these authors had reported the absence of estrogen-like effects in the maturing reproductive tracts of male quail administered up to 1000 ppm atrazine (Wilhelms et al. 2005). Finally, a study by LeGoff and coworkers (2006) showed that atrazine did not induce the expression of yolk protein genes in *Drosophila* incubated on atrazine-containing medium, whereas estrogen was capable of inducing these genes.

In some studies, high doses of atrazine antagonized estrogen-mediated responses. For example, Tennant et al. (1994a) observed diminished uterine weight, diminished progesterone receptor expression, and diminished thymidine incorporation into uterine DNA in estrogen-treated OVX rats administered atrazine. Connor et al. (1996) reported a similar antagonism of the estrogen-mediated response in rat uterus. The concentration necessary to elicit the antagonistic responses were typically very high, at least 10^6 times greater than the picomolar levels of estradiol needed to activate the estrogen receptor.

In contrast, a more recent study by McMullin et al. (2004) showed that atrazine did not block the estrogen-stimulated expression of mRNA that codes for the progesterone receptor. Sanderson et al. (2001) reported that carp exposed to atrazine did not display any effect on estrogen-stimulated vitellogenin production. In addition, the study by Wilhelms et al. (2005) on immature Japanese quail administered atrazine in the diet, and the study by LeGoff et al. (2006) on atrazine-exposed fruit flies, found no evidence of an effect of atrazine on naturally occurring estrogen-mediated responses.

Another well-studied estrogen-specific *in vivo* response in rodents is the ability of estrogen-primed OVX female rats to produce a daily surge of pituitary LH.

Administration of atrazine, for as short as 3 d, or as long as 6 mon, has been found to inhibit these estrogen-primed LH surges (Simpkins et al. 1998; Eldridge et al. 1999a; Cooper et al. 2000; McMullin et al. 2004). The successful generation of the light-entrained, estrogen-primed LH surge in rodents is dependent upon a cascade of several nonestrogen neuroendocrine components, any of which may be blocked by atrazine. Although possible, it is unlikely that atrazine antagonism of the estrogen receptor plays a role in the suppression of LH release.

In summary, from these *in vivo* studies, which employed a wide variety of well-recognized, standard, and specific biological responses to estrogen, it can be concluded that atrazine does not elicit estrogen-like responses, even at dose levels up to a million-fold greater than the minimally effective estrogen dose. These results support the conclusion that atrazine is not an estrogen receptor agonist.

In some of the previously described models, however, high doses of atrazine appeared to inhibit or reduce the response to estrogen. This "inhibition" typically occurs at atrazine doses near to, or greater than, the MTD, and at levels several orders of magnitude greater than the amount of estrogen required to initiate the response. Therefore, one can conclude, from the foregoing review, that atrazine antagonism of estrogen-mediated responses *in vivo* is either nonexistent or extremely weak, and is unlikely to be relevant to man under conditions of potential human exposure (US EPA 2006).

3.2 In Vitro Expression Systems

Table 2 presents an overview, taken from the literature, of responses to atrazine exposure during testing of estrogen expression *in vitro*.

A number of investigators have evaluated the interaction of atrazine with estrogen expression by using constructs containing an estrogen receptor (ER) and a reporter composed of an easy-to-measure cellular response, or with a reporter gene tethered to a naturally occurring estrogen-activated receptor-dependent genomic site. These *in vitro* models are relatively easy to run, highly precise and specific, and they permit assessment of both agonist and antagonist potential within the same system.

Atrazine has consistently failed to activate estrogen-dependent reporters *in vitro* in estrogen-dependent expression systems. Atrazine failed to stimulate estrogen-dependent MCF-7 cell proliferation (Soto et al. 1995; Connor et al. 1996; Fukamachi et al. 2004); and atrazine did not enhance an estrogen-induced increased aggregation of the progesterone receptor-progesterone response element (PR-PRE) derived from nuclear DNA extracts of MCF-7 cells (Connor et al. 1996). Estrogen-dependent proliferation of transfected yeast cells (Connor et al. 1996) and MtT/E-2 cells (Fujimoto and Honda 2003) did not occur upon incubation with atrazine. Similarly, production of specific estrogen-mediated products did not respond to atrazine in reporter constructs of MCF-7 cells (Connor et al. 1996; Balaguer et al. 1996), in HeLa cells transfected with the alpha- or beta-subunit forms of ER (Balaguer et al. 1996),

Table 2 Responses to atrazine exposure using *in vitro* tests of estrogen expression

Cell type	Test	Stimulated [a]	Inhibited [a,b]	Reference
MCF-7	ER-mediated proliferation	No	No	Connor et al. 1996
MCF-7	ER-mediated proliferation	No	—	Soto et al. 1995
MCF-7	ER-mediated proliferation	No	—	Fukamachi et al. 2004
MCF-7	Nuclear DNA—PgR complex	No	No	Connor et al. 1996
MCF-7	ER-mediated genetic expression	No	No	Connor et al. 1996
MCF-7	ER-mediated genetic expression	No	No	Balaguer et al. 1996
HeLa	ER-mediated genetic expression	No	—	Balaguer et al. 1996
HeLa	ER-α- and ER-β-mediated expression	No	—	Balaguer et al. 1996
CHO cells	ER-α- and ER-β-mediated expression	No	—	Kojima et al. 2004
T47D.Luc	ER-mediated genetic expression	No	—	Legler et al. 2002
MtT/E-2	ER-mediated proliferation	No	—	Fujimoto and Honda 2003
Yeast	ER-mediated proliferation	No	No	Connor et al. 1996
Yeast	ER-mediated genetic expression	No	Weak	Tran et al. 1996
Yeast	ER-mediated genetic expression	No	No	Graumann et al. 1999
Yeast	ER-mediated genetic expression	No	—	O'Conner et al. 2000
Clam gills	Metabolic stimulation	No	No	Cheney et al. 1997
Fish. hepatic	Vitellogenin production	No	Weak	Sanderson et al. 2001

ER, estrogen receptor; —, assessment not done.

[a] No, indicates no response to atrazine was observed at doses that were at least 10^5 in excess of the effective dose of estradiol.

[b] Weak, indicates a response to atrazine was observed only at doses that were at least 10^5 in excess of the effective dose of estradiol.

in yeast cells (Tran et al. 1996; Graumann et al. 1999; O'Connor et al. 2000), or in T47D Luc cells (Legler et al. 2002).

Cheney and coworkers (1997) measured metabolic stimulation of clam gills incubated *in vitro* with atrazine, and Sanderson et al. (2001) incubated fish hepatic cells with atrazine; neither study produced evidence of an atrazine-induced "estrogen-like response." Other laboratories have tested for atrazine antagonism of estrogen stimulation in a transfected construct. Connor et al. (1996) reported that atrazine coincubated with estradiol failed to inhibit an ER-mediated proliferation of MCF-7 cells. Atrazine also failed to inhibit estrogen-mediated formation of a progesterone receptor complex with DNA, and showed a similar failure with ER-mediated genetic expression in MCF-7 cells (Connor et al. 1996). Balaguer et al. (1999) confirmed that atrazine did not inhibit ER-mediated expression in MCF-7 cells.

In studies of transfected yeast cells, Connor et al. (1996) found that atrazine did not block estrogen-mediated cell proliferation, and Graumann et al. (1999) reported that atrazine did not inhibit an ER expression system transfected into yeast. This latter result contrasted with earlier findings of Tran et al. (1996), who observed a weak inhibition by atrazine of an estrogen-mediated reporter in yeast cells. Atrazine was unable to block estrogen stimulation of clam gill metabolism (Cheney et al. 1997), but it weakly inhibited vitellogenin production by fish liver cells incubated *in vitro* (Sanderson et al. 2001).

To summarize the results from *in vitro* studies, a variety of tests using well-established, estrogen-dependent reporter systems have uniformly failed to demonstrate an ability of atrazine to imitate the action of estrogen. Moreover, a majority of tests of estrogen antagonism by atrazine also produced negative findings. We conclude from them foregoing that atrazine does not interact with the estrogen receptor in these incubation systems.

3.3 Estrogen Receptor Binding

Steroid hormones, such as estrogen, serve as an activating ligand for specific intracellular receptors (ER-α or ER-β). Upon activation, these hormone-bound proteins are attracted to specific chromatin sites. Once bound to DNA, the hormone–ligand complex serves to attract additional factors that initiate and maintain mRNA transcription. Although binding of a particular ligand to the estrogen receptor may not necessarily enhance or diminish an estrogen-mediated response, it is typically assumed that receptor interaction is mandatory for classic estrogen-mediated expression to occur. Many investigators have studied whether atrazine may interact directly with the estrogen receptor.

Table 3 presents a summary, from the literature, of studies in which there was atrazine competition against estrogen receptor binding in yeast or various animal tissues. Tennant et al. (1994b) reported that competitive coincubation of atrazine and radiolabeled estrogen with ER-containing rat uterine cytosol failed to produce significant displacement of estrogen binding by atrazine. However, when uterine cytosols were preincubated with atrazine, and a tritium tracer was later added to a chilled incubation, there was a significant reduction of [^{3}H]estradiol association. Scatchard analysis suggested a very weak competitive antagonism at concentrations that were several orders of magnitude greater than the subnanomolar disassociation constant (K_d) for estradiol binding to ER. Thomas and Dong (2006) recently reported that atrazine very weakly displaced estradiol binding to a seven-transmembrane receptor (GPR30) stably transfected into HEK-293 cells. These results indicate that, when atrazine is allowed to compete with estrogen for the estrogen receptor, no antagonism is found. However, when high concentrations of atrazine are preincubated with the estrogen receptor before the addition of estradiol, weak antagonism can be observed.

Tennant et al. (1994b) also showed that, when radiolabeled estradiol binding was assessed in uterine cytosols prepared from OVX rats orally dosed with atrazine

Table 3 Atrazine competition against estrogen receptor (ER) binding in various tissues or organisms

ER source	Incubation	Competition[a,b]	Reference
Rat uterus	*In vitro*	Weak	Tennant et al. 1994b
Rat uterus	*In vitro*	Weak	McMullin et al. 2004
Rat uterus	*In vitro*	No	Danzo 1997
Rat uterus	*In vitro*	No	O'Connor et al. 2000
Rat uterus	*Ex vivo*	Weak	Tezak et al. 1992
Rat uterus	*Ex vivo*	Weak	Tennant et al. 1994b
Rat hypothalamus	*Ex vivo*	No	McMullin et al. 2004
Human ER-α and -β	*In vitro*	No	Roberge et al. 2004
Human ER	*In vitro*	Weak	Hanoika et al. 1999
Human ER-α	*In vitro*	Weak	Scippo et al. 2004
Transfected yeast	*In vitro*	Weak	Tran et al. 1996
Alligator oviduct	*In vitro*	Weak	Vonier et al. 1996
ER GPR30 in HEK293 cells	*In vitro*	Weak	Thomas and Dong 2006

[a] Weak, indicates negative inhibition at concentrations of at least 10^5 molar excess.
[b] No, indicates no inhibition at concentrations of at least 10^5 molar excess.

before being killed, a similar reduction of radioligand association with its receptor was observed. This latter finding confirmed earlier work by Tezak et al. (1992). McMullin and coworkers (2004) observed a mild competition in rat hypothalamus between atrazine and estrogen for the estrogen receptor from orally administered atrazine. In contrast, other groups have not observed significant competition by atrazine for rat uterine ER binding *in vitro* (Danzo 1997; O'Connor et al. 2000; McMullin et al. 2004).

Hanioka et al. (1999) and Scrippo et al. (2004), however, using a noncellular preparation containing recombinant human ER-α, observed a very weak competition for receptor binding by atrazine and estrogen, although Roberge et al. (2004) were unable to observe significant atrazine displacement of estradiol binding to incubated recombinant human ER-α or ER-β. Vonier and coworkers (1996) also reported that atrazine demonstrated limited competition against estradiol binding to cytosols prepared from alligator oviduct. Finally, Tran et al. (1996) observed very weak displacement of estradiol in yeast cells transfected with the estrogen receptor.

In conclusion, there is evidence that atrazine has a limited capacity to antagonize the binding of estrogen to its receptors. Antagonism of estrogen binding to ER is not expected to occur under equilibrium conditions, or at concentrations expected as a result of normal human exposure.

4 Summary

More than 40 publications have described results of atrazine responses in 17 estrogen-dependent systems and in more than a dozen different reporter and estrogen receptor-binding studies *in vitro*. Results from these studies have consistently failed to

demonstrate that atrazine acts as an estrogen agonist. Moreover, a variety of indices of estrogen-dependent activity, in models that encompass cell incubations to whole animals, have failed to respond to atrazine. Researchers in more than a dozen laboratories have examined rats, rat tissues, human and prokaryotic cells, in addition to tissues from reptile, fish, amphibian, avian, molluscan, and insect sources, without eliciting estrogenic-like responses from atrazine.

In contrast, studies of atrazine ability to antagonize estrogen-mediated responses have yielded equivocal results. Results of several studies show inhibition of estrogen-like activities by atrazine, yet many other tests have yielded negative results. Generally, *in vivo* models have more consistently shown that atrazine inhibits estrogen-mediated responses, whereas in more specific *in vitro* systems, inhibition is seldom observed. The implication is that *in vivo* effects of atrazine may result from inhibition of factors that are indirectly connected to the genomic interaction of estrogen (e.g., at the receptor). Potential targets of atrazine may be downstream of the ligand–receptor binding event. Atrazine may also interact with other, less specific, factors that are necessary for the completion of the estrogen-mediated response.

Moreover, the apparent inhibition of cytosolic-ER binding by atrazine may, similarly, be relatively nonspecific. Observed inhibitory responses occur only at extreme doses or concentrations, i.e., several orders of magnitude greater than the level of estradiol presence in each test system. It is probable that the inhibitory effects result from very low affinity and/or low specificity interactions, which are unlikely to occur in nature.

We conclude that atrazine is not an estrogen receptor agonist, but it may be a weak antagonist, when present at a high concentration under conditions of disequilibrium with estrogen. These conditions are not expected to occur as a result of normal environmental exposure.

References

APVMA (Australian Pesticides and Veterinary Medicines Authority) (2004) The reconsideration of approvals of the active constituent atrazine, registrations of products containing atrazine, and their associated labels. Second Draft Final Review Report Including Additional Assessments. October 2004. Australian Pesticides and Veterinary Medicines Authority, Canberra, Australia.

Ashby J, Tinwell H, Stevens J, Pastoor T, Breckenridge CB (2002) The effects of atrazine on the sexual maturation of female rats. Regul Toxicol Pharm 35:468–473.

Balaguer P, Joyeux A, Denison MS, Vincent R, Gillesby BE, Zacharewski T (1996) Assessing the estrogenic and dioxin-like activities of chemicals and complex mixtures using in vitro recombinant receptor-reporter gene assays. Can J Physiol Pharmacol 74:216–222.

Balaguer P, Francois F, Comunale F, Fenet H, Boussioux AM, Pons M, Nicolas JC, Casellas C (1999) Reporter cell lines to study the estrogenic effects of xenoestrogens. Sci Total Environ 233:47–56.

Beldomenico PM, Rey F, Prado WS, Villarreal JC, Munoz-de-Toro M, Luque EH (2007) *In ovum* exposure to pesticides increases the egg weight loss and decreases hatchlings weight of *Caiman latirostris* (Crocodylia: Alligatoridae). Ecotoxicol Environ Saf 68:246–251.

Bringolf RB, Belden JB, Summerfelt RC (2004) Effects of atrazine on fathead minnow in a short-term reproduction assay. Environ Toxicol Chem 23:1019–1025.

Calle EE, Frumkin H, Henley SJ, Savitz DA, Thun MJ (2002) Organochlorines and breast cancer risk. CA Cancer J Clin 52:301–309.

Carr JA, Gentiles A, Smith EE, Goleman WL, Urquidi LJ, Thuett K, Kendall RJ, Giesy JP, Gross TS, Solomon KR, Van Der Kraak G (2003) Response of larval *Xenopus laevis* to atrazine: assessment of growth, metamorphosis, and gonadal and laryngeal morphology. Environ Toxicol Chem 22:396–405.

Cheney MA, Fiorillo R, Criddle RS (1997) Herbicide and estrogen effects on the metabolic activity of *Elliptio complanata* measured by calorespirometry. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 118:159–164.

Conner K, Howell J, Chen I, Liu H, Berhane K, Sciarretta C, Safe S, Zacharewski T (1996) Failure of chloro-s-triazine-derived compounds to induce estrogen receptor-mediated responses *in vivo* and *in vitro*. Fundam Appl Toxicol 30:93–101.

Cooper RL, Stoker TE, Tyrey L, Goldman JM, McElroy WK (2000) Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicol Sci 53:297–307.

Crain DA, Spiteri ID, Guillette LJ Jr (1999) The functional and structural observations of the neonatal reproductive system of alligators exposed *in ovo* to atrazine, 2,4-D, or estradiol. Toxicol Ind Health 15:180–185.

Danzo B (1997) Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environ Health Perspect 105:294–301.

Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H (1993) Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ Health Perspect 101:372–377.

De Solla SR, Martin PA, Fernie KJ, Park BJ, Mayne G (2006) Effects of environmentally relevant concentrations of atrazine on gonadal development of snapping turtles (*Chelydra serpentine*). Environ Toxicol Chem 25:520–526.

Devine MD, Duke SO, Fedtke C (1993) Herbicidal inhibition of photosynthetic electron transport. In: Physiology of Herbicide Action. Prentice-Hall, Englewood Cliffs, NJ, pp 113–140.

Eldridge JC, Tennant MK, Wetzel LT, Breckenridge CB, Stevens JT (1994) Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: hormonal properties, dosage and animal strain. Environ Health Perspect 102(suppl 11):29–36.

Eldridge JC, McConnell RF, Wetzel LT, Tisdel, MO (1998) Appearance of mammary tumors in atrazine-treated female rats: probable mode of action involving strain-related control of ovulation and estrous cycling. In: Ballantine LG, McFarland JE, Hackett DS (eds) Triazine Herbicides: Risk Assessment. Oxford University Press, Washington, DC, pp 414–423.

Eldridge JC, Wetzel LT, Stevens JT, Simpkins JW (1999a) The mammary tumor response in triazine-treated female rats: a threshold-mediated interaction with strain and species-specific reproductive senescence. Steroids 64:672–678.

Eldridge JC, Wetzel LT, Tyrey L (1999b) Estrous cycle patterns of Sprague–Dawley rats during acute and chronic atrazine administration. Reprod Toxicol 13:491–499. European Union (2000) Atrazine. Volume 3. Annex B. Addendum to the Report and Proposed Decision of the United Kingdom Made to the European Commission under Article 7(1) of Regulation 3600/92. Summary, Scientific Evaluation and Assessment. Council Directive 91/414/EEC Regulation 3600/92. February 2000.

Fujimoto N, Honda H (2003) Effects of environmental estrogenic compounds on growth of a transplanted estrogen responsive pituitary tumor cell line in rats. Food Chem Toxicol 41:1711–1717.

Fukamachi K, Han BS, Kim CK, Takasuka N, Matsuoka Y, Matsuda E, Yamasaki T, Tsuda H. (2004) Possible enhancing effects of atrazine and nonylphenol on 7,12-dimethylbenz[*a*]anthra cene induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. Cancer Sci 95:404–410.

Good NE (1961) Inhibitors of the Hill reaction. Plant Physiol 36:788–803.

Graumann K, Breithofer A, Jungbauer A (1999) Monitoring of estrogen mimics by a recombinant yeast assay: synergy between natural and synthetic compounds? Sci Total Environ 225:69–79.

Hanioka N, Jinno H, Tanaka-Kagawa T, Nishimura T, Ando M (1999) *In vitro* metabolism of simazine, atrazine and propazine by hepatic cytochrome P450 enzymes of rat, mouse and guinea pig, and oestrogenic activity of chlorotriazines and their main metabolites. Xenobiotica 29:1213–1226.

Hauswirth JW, Wetzel LT (1998) Toxicity characteristics of the 2-chlorotriazines atrazine and simazine. In: Ballantine LG, McFarland JE, Hackett DS (eds) Triazine Herbicides: Risk Assessment. Oxford University Press, Washington, DC, pp 370–383.

Hayes TB (2005) Atrazine and pesticide mixtures: sum of the parts or some of the parts? In: SETAC Annual Meeting, Baltimore, MD, USA. SETAC, Pensacola, FL.

Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, Vonk A (2002) Hermaphoditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci U S A 99(8):5476–5480.

Hayes TB, Stuart AA, Mendoza M, Collins A, Noriega N, Vonk A, Johnston G, Liu R, Kpodzo D (2006) Characterization of atrazine-induced gonadal malformations in African clawed frogs (*Xenopus laevis*) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17-estradiol): support for the demasculinization/feminization hypothesis. Environ Health Perspect 114(1):134–141.

IARC (1999) Atrazine. Some Chemicals That Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. World Health Organization International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Views and Expert Opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon, 13–20 October 1998, 33:59–113.

Kloas W, Lutz I, Springer T, Krueger H, Wolf J, Holden L, Hosmer A (2008) Atrazine does not induce gonadal feminization in *Xenopus laevis*. Submitted to Proc Natl Acad Sci U S A.

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004) Screening for estrogen and androgen receptor activities in 200 pesticides by *in vitro* reporter gene assays using Chinese hamster ovary cells. Environ Health Perspect 112:524–531.

Legler J, Dennenkamp M, Vethaak AD, Brouwer A, Koeman JH, van der Burg B, Murk AJ (2002) Detection of estrogenic activity in sediment-associated compounds using *in vitro* reporter gene assays. Sci Total Environ 293:69–83.

LeGoff G, Hilliou F, Siegfried BD, Boundy S, Wajnberg E, Sofer L, Audant P, French-Constant RH, Feyereisen R (2006) Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and GST gene induction. Insect Biochem Mol Biol 36:674–682.

McMullin TS, Andersen ME, Nagahara A, Lund TD, Pak T, Handa RJ, Hanneman WH (2004) Evidence that atrazine and diaminochlorotriazine inhibit the estrogen/progesterone induced surge of luteinizing hormone in female Sprague–Dawley rats without changing estrogen receptor action. Toxicol Sci 79:278–286.

Mitra AK, Faruque FS, Avis AL (2004) Breast cancer and environmental risk: where is the link? J Environ Health 66:24–32.

Muir K, Rattanamongkolgul S, Smallman-Raynor M, Thomas M, Downer S, Jenkinson C (2004) Breast cancer incidence and its possible spatial association with pesticide application in two counties of England. Public Health 118:513–520.

Mukherjee S, Koner BC, Ray S, Ray A (2006) Environmental contaminants in pathogenesis of breast cancer. Indian J Exp Biol 44:597–617.

Muncke J, Junghans M, Eggen RIL (2007) Testing estrogenicity of known and novel (xeno-) estrogens in the MolDarT using developing zebrafish (*Danio rerio*). Environ Toxicol 22:185–193.

O'Connor JC, Plowchalk DR, Van Pelt CS, Davis LG, Cook JC (2000) Role of prolactin in chloro-*s*-triazine rat mammary tumorigenesis. Drug Chem Toxicol 23:575–601.

Qiao X, Ma L, Hummel HE (1996) Persistence of atrazine and occurrence of its primary metabolites in three soils. J Agric Food Chem 44:2846–2848.

Roberge M, Hakk H, Larsen G (2004) Atrazine is a competitive inhibitor of phosphodiesterase but does not affect the estrogen receptor. Toxicol Lett 154:61–68.
Safe S (2004) Endocrine disruptors and human health: is there a problem? Toxicology 205:3–10.
Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M (2001) Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ Health Perspect 109:1027–1031.
Schottler SP, Eisenreich SJ, Hines NA, Warren G (1998) Temporal and spatial trends of atrazine, desethylatrazine, and desisopropylatrazine in the Great Lakes. In: Ballantine LG, McFarland JE, Hackett DS (eds) Triazine Herbicides: Risk Assessment. Oxford University Press, Washington, DC, pp 208–226.
Scippo ML, Argiris C, Van De Weerdt C, Muller M, Willemsen P, Martial J, Maghuin-Rogister G (2004) Recombinant human estrogen, androgen and progesterone receptors for detection of potential endocrine disruptors. Anal Bioanal Chem 378:664–669.
Simpkins JW, Eldridge JC, Wetzel LT (1998) Role of strain-specific reproductive patterns in appearance of mammary tumors in atrazine treated rats. In: Ballantine LG, McFarland JE, Hackett DS (eds) Triazine Herbicides: Risk Assessment. Oxford University Press, Washington, DC, pp 399–413.
Sonnenschein C, Soto AM (1998) An updated review of environmental estrogen and androgen mimics and antagonists. J Steroid Biochem Mol Biol 65:143–150.
Soto AM, Sonnenschein C, Chung KL, Fernandez MF, Olea N, Serrano FO (1995) The E-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. Environ Health Perspect 104(suppl 7):113–122.
Spano L, Tyler CR, van Aerle R, Devos P, Mandiki SN, Silvestre F, Thome JP, Mestemont P (2004) Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonadal development in adult goldfish (*Carassius auratus*). Aquat Toxicol 66:369–379.
Steingraber S (1997) Mechanisms, proof, and unmet needs: the perspective of a cancer activist. Environ Health Perspect 105(suppl 3):685–687.
Stevens JT, Breckenridge CB, Wetzel L (1999) A risk characterization for atrazine. J Toxicol Environ Health 56:69–109.
Tanaka T, Kohno H, Suzuki R, Sugie S (2004) Lack of modifying effects of an estrogenic compound atrazine on 7,12-dimethylbenz(*a*)anthracene-induced ovarian carcinogenesis in rats. Cancer Lett 210:129–137.
Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB, Stevens JT (1994a) Possible antiestrogenic properties of chloro-*s*-triazines in rat uterus. J Toxicol Environ Health 43:183–196.
Tennant MK, Hill DS, Eldridge JC, Wetzel LT, Breckenridge CB, Stevens JT (1994b) Chloro-*s*-triazine antagonism of estrogen action: limited interaction with estrogen receptor binding. J Toxicol Environ Health 43:197–211.
Tezak Z, Simi B, Kniewald J (1992) Effect of pesticides on oestradiol-receptor complex formation in rat uterus cytosol. Food Chem Toxicol 30:879–885.
Thomas P, Dong J (2006) Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. J Steroid Biochem Mol Biol 102:175–179.
Tran DQ, Kow KY, McLachlan JA, Arnold SF (1996) The inhibition of estrogen receptor-mediated responses by chloro-*s*-triazine-derived compounds is dependent on estradiol concentration in yeast. Biochem Biophys Res Commun 227:140–146.
United Kingdom Pesticide Directorate (2000) Atrazine: Report and Proposed Decision of the United Kingdom Made to the European Commission Under Article 7(1) of Regulation 3600/92 Council Directive 91/4 14/EEC Regulation 3600/92, UK. Rapporteur Monograph, October 1996.
US EPA (1996) Drinking water regulation and health advisories. EPA 822-B-96-002. Office of Water, U.S. Environmental Protection Agency, Washington, DC.
US EPA (2002) Atrazine Toxicology Chapter of the Re-registration Eligibility Decision, Second Revision, April 11, 2002.

US EPA (2006) Triazine Cumulative Risk Assessment. HED Human Health Risk Assessment in Support of the Reregistration Eligibility Decisions for Atrazine, Simazine and Propazine. PC Codes: 080808, 080803, 080807. DP 317976. Health Effects Division (7509C), Benefits and Economic Analysis Division (7503C), and Environmental Fate and Effects Division (7507C). March 28, 2006. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency, Washington, DC.

Vencill WK (2002) Herbicide Handbook, 8th Ed. Weed Science Society of America, Lawrence, KS, pp 493–494.

Vonier PM, Crain DA, McLachlan JA, Guillette LJ Jr, Arnold SF (1996) Interaction of environmental chemicals with the estrogen and progesterone receptors from the oviduct of the American alligator. Environ Health Perspect 104:1318–1322.

Wetzel LT, Luempert LG III, Breckenridge CB, Tisdel MO, Stevens JT, Thakur AK, Extrom PJ, Eldridge JC (1994) Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer-344 rats. J Toxicol Environ Health 43:169–182.

Wilhelms KW, Cutler SA, Proudman JA, Anderson LL, Scanes CG (2005) Atrazine and the hypothalamo-pituitary-gonadal axis in sexually maturing precocial birds: studies in the male Japanese quail. Toxicol Sci 86:152–160.

Wilhelms KW, Cutler SA, Proudman JA, Carsia RV, Anderson LL, Scanes CG (2006) Lack of effects of atrazine on estrogen-responsive organs and circulating hormone concentrations in sexually immature female Japanese quail (*Coturnix coturnix japonica*). Chemosphere 3:64

Winkelmann DA, Kliane SJ (1990) Degradation and bound residue formation of urof atrazine metabolites, deethylatrazine, dealkylatrazine and hydroxyatrazine, in a western Tennessee soil. Environ Toxicol Chem 10(3):347–354.