## SUPPLEMENTARY TEXT AND TABLES

### Justification for Excluding Studies from the Meta-analysis

*Metamorphosis*

For our meta-analysis on effects of atrazine on amphibian metamorphosis, six studies were excluded (Table 1 & S1). La Fiandra et al. (2008) was excluded from our meta-analyses because substantial concentrations of several triazine pesticides were detected in their well water (negative) "control". Allran and Karasov (2000) was excluded for timing of metamorphosis because only used 50% of the metamorphs were used in this statistical analysis without describing how they selected this subset of metamorphs or why they only used 50% when 100% of the metamorphs were used for the size at metamorphosis analysis. Orton et al. (2006) was excluded for timing of metamorphosis because they claimed that there was no significant effect of atrazine on this trait but also provided a probability value less than 0.05. No data, test statistics, or within-group variance estimates were provided to assess whether the effect was likely significant or not. Three studies (Kloas et al. 2009, Oka et al. 2008, and Hayes et al. 2002b) were excluded because they did not provide sufficient statistical information (e.g., test statistics or degrees of freedom) or data (means and variances) making it impossible for us to evaluate their findings (Table S1). La Fiandra (2008) and Orton et al. (2006) had unknown effects on metamorphosis and the four remaining studies claimed to have not detected effects of atrazine on the focal metamorphic trait (Table S1).

*Behavior*

Only Koprivnikar et al. (2007) was excluded from our meta-analyses on behavior because no variance estimates were provided for any response variable in this paper (Table S1). These authors did not find effects of atrazine on behavior.

*Immunity and Infections*

Cossariniduniер (1987) was the only study excluded from the meta-analysis on immunity (Table S1). It was excluded because they only studied cells from a single fish, and provided no statistics or within-group variance estimates. Even in this study, at maximum chemoluminescence, every atrazine concentration tested resulted in less macrophage phagocytosis than the controls (Cossariniduniер 1987).

Four studies were not included in our meta-analysis on the effects of atrazine on infections because they had insufficient power, obvious confounders, or inadequate statistics (Table S1). Griggs and Belden (2008) was excluded for several reasons, including experimental design issues and solvent concentrations that affected cercariae (Table S1). This study had insufficient statistical power because they used cercariae that were moribund (10 h post-shedding). For example, across all treatments where cercariae were exposed to atrazine, an average of 5% infected *Rana clamitans* tadpoles, whereas infection rates for cercariae in the literature are typically between 20% and 90% (Kiesecker 2002; Koprivnikar et al. 2007; Rohr et al. 2008b). The tadpoles and cercariae in this study were also only exposed to atrazine for 10 h. This is not an ecologically relevant exposure and unlikely enough time for atrazine to alter the abundance of amphibian immune cells because the half-life for amphibian eosinophils, basophils, and neutrophils is up to 8 h, whereas the half-life for lymphocytes is 3-8 weeks (Raffel et al. 2006). Finally, the mesocosm study conducted by Griggs and Belden (2008) confounded

tadpole mortality with trematode loads and thus we do not know if the lack of an effect of atrazine was because the most infected individuals died or because there was no effect of atrazine.

Koprivnikar et al. (2006a) was excluded because only one of 12 sampled ponds had concentrations of atrazine that were above the method detection limit, providing insufficient statistical power to detect any effects of atrazine on parasite abundance (Table S1). King et al. (2007) was not included because it was spatially confounded. Wetlands within each of the pesticide categories were much closer to one another than they were to any wetlands within the other categories, making it impossible to know whether any significant effects of atrazine were because of the pesticides or something else about each spatial region. Finally, we excluded Koprivnikar et al. (2007) because no variance estimates were provided for any response variable. Of the four studies excluded from the infection meta-analysis, three did not detect effects of atrazine while one detected elevated infections if the frogs were exposed to atrazine (Table S1).

*Gonadal Morphology*

Twelve studies were excluded from our meta-analysis on general gondal gross morphology (Table S1). Hayes et al. (2003; 2002b) and Kloas et al. (2009) were excluded because they draw conclusions without presenting statistical analyses, results (e.g. test statistics), or data (e.g., means). Tavera-Mendoza et al. (2002a; 2002b), Spanò et al. (2004) and Nadzialek et al. (2008) were excluded because they were pseudoreplicated (Table S1); that is, they used individuals within tanks, rather than the tank, as the replicate. Hecker et al. (2004) and Coady et al. (2004; 2005) were excluded because reference sites or negative controls were contaminated with atrazine, and Jooste et al. (2005) and Orton et al. (2006) were excluded due to high background

levels of gonadal abnormalities that occurred in their control treatments (Table S1). One of the eleven excluded studies found no significant effects of atrazine, four report significant effects, and six have unknown effects due to contaminated control or reference populations or atypically high quantities of gonadal abnormalities in control treatments (Table S1).

*Sex Ratios*

Four studies were excluded from our meta-analysis on sex ratios (Table S1). Suzawa and Ingraham, (2008) did not present statistical methodology or results (e.g. test statistics, degrees of freedom, p-values), Coady et al. (2004; 2005) had contaminated negative controls as well as statistical errors, and Orton et al. (2006) had unusually high background levels of gonadal abnormalities (12% intersex individuals- having both male and female gonadal tissue) in control treatments [compare to (Carr et al. 2003a; Hayes et al. 2002b; Kloas et al. 2009; Oka et al. 2008) which all report occurrences lower than 1%, Table 6]. One of these studies, the only one conducted on fish, reports significant effects of atrazine (Suzawa and Ingraham 2008), and three have unknown effects due to contaminated control populations or abnormally high quantities of gonadal abnormalities in control treatments.

*Gonadal Function*

Six studies were excluded from our meta-analysis on testicular cell types (Table S1). Four studies were pseudoreplicated (Spano et al. 2004; Tavera-Mendoza et al. 2002a) or presented no statistics (Bringolf et al. 2004; Kloas et al. 2009). One study (Smith et al. 2005) had atrazine contaminated reference sites (reported in Hecker et al. 2004), and one had unusually high levels of gonadal abnormalities in the control treatment (Orton et al. 2006)(Table S1). Two of these six

studies found effects of atrazine on spermatogenesis, two had unknown effects, and two reported no effects.

Four studies were excluded from our meta-analysis on sex hormone concentrations (Table S1). Spanò et al. (2004), Salaberria et al. (2009) and Nadzialek et al. (2008) were psuedoreplicated, whereas Coady et al. (2005) had atrazine contaminated controls (up to 0.29 µg/L ; Table S1). Three of the four excluded studies report significant effects of atrazine on sex hormone concentrations.

Du Preez et al. (2008) was excluded from our discussion of the effects of atrazine on reproductive success (Table S1) because control animals (those from Jooste et al. 2005) had unusually high background levels of gonadal abnormalities (~50% had testicular ovarian follicles-see Table S1).

*Vitellogenin*

Two studies were excluded from our meta-analysis because they were psuedoreplicated (Salaberria et al. 2009; Spano et al. 2004) (Table S1). Spanò et al. (2004) found no effects of atrazine. Salaberria et al. (2009), however, did find a significant effect of atrazine on plasma vitellogenin concentrations, but the route of exposure was via an intraperitoneal injection and not through aqueous exposure, as was the case for all other studies either included or excluded from this vitellogenin analysis. Indeed, aqueous exposures are more ecologically relevant, so including this study in our analysis does not change our conclusion that atrazine, at ecologically relevant doses, does not appear to be estrogenic.

*Aromatase*

Two studies were excluded from this meta-analysis on aromatase (Table S1). One (Hecker et al. 2004) had atrazine-contaminated reference sties and the other was pseudoreplicated (Nadzialek et al. 2008). Both of these studies report no effects of atrazine on aromatase activity or expression.

**Literature Cited in Supplementary Materials**


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**Table S1.** Endpoints excluded from the meta-analysis.

| Taxon | Species | Endpoint | Effect direction | Conc. where effect was observed (µ/L) | Conc. tested (µ/L) | Atrazine grade | Experi-ment type[bb] | Exposure duration[a] | Excluded from meta-analysis? | Reference | Response |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Frog | *Hyla versicolor* | Net effect on developmental rate | None, trend toward increase | - | 20, 200 | Commercial; Aatrex | SR | Until fore-limb emerg-ence, about 50 | Yes | LaFiandra et al. 2008[b,c] | Net effect on developmental rate |
| Frog | *Hyla versicolor* | Size at or near metamorphosis | None detected | - | 20,200 | Commercial; Aatrex | SR | Until fore-limb emerg-ence, about 50 | Yes | LaFiandra et al. 2008[b,c] | Size at or near metamorphosis |
| Frog | *Rana pipiens* | Net effect on developmental rate | Unknown | - | 10 | Technical | SR | LTM | Yes | Orton et al. 2006[d,e] | Net effect on developmental rate |
| Frog | *Rana pipiens* | Size at or near metamorphosis | Unknown | - | 10 | Technical | SR | LTM | Yes | Orton et al. 2006[f,g] | Size at or near metamorphosis |
| Frog | *Xenopus laevis* | Net effect on developmental rate | None detected | - | 0.1, 1, 10, 25 | Technical | SR | 185 d or less | Yes | Coady et al. 2005[h] | Net effect on developmental rate |
| Frog | *Xenopus laevis* | Size at or near metamorphosis | Unknown | - | 0.1, 1, 10, 26 | Technical | SR | 186 d or less | Yes | Coady et al. 2005[h] | Size at or near metamorphosis |
| Frog | *Xenopus laevis* | Net effect on developmental rate | None detected | - | 0.01, 0.1, 1, 10, 25; 0.1, 0.4, 0.8, 1.0, 25, 200 | Technical | SR | LTM | Yes | Hayes et al. 2002b[h] | Net effect on developmental rate |
| Frog | *Xenopus laevis* | Size at or near metamorphosis | None detected | - | 0.01, 0.1, 1, 10, 25; 0.1, 0.4, 0.8, 1.0, 25, 201 | Technical | SR | LTM | Yes | Hayes et al. 2002b[h] | Size at or near metamorphosis |
| Frog | *Xenopus laevis* | Net effect on developmental rate | Unknown | - | 0.1, 1, 10, 100 | Technical | SR | LTM | Yes | Oka et al. 2008[e,g] | Net effect on developmental rate |
| Frog | *Xenopus laevis* | Size at or near metamorphosis | Unknown | - | 0.1, 1, 10, 100 | Technical | SR | LTM | Yes | Oka et al. 2008[e,g] | Size at or near metamorphosis |
| Frog | *Rana sylvatica* | Locomotor activity | None detected | - | 3, 30 | Commercial; Aatrex | SR | 31 d for tadpoles, 1h for cercariae | Yes | Koprivnikar et al. 2007[i,,j] | Amphibian Behaviors; Locomotor Activity |
| Fish | *Cyprinus caprio* | Chemoluminescence of kidney and spleen macrophages phagocytosing opsonized zymosan | No effect, but decrease in every conc. relative to control at max. chemo- | - | 7000-28000 | Technical | PE | 2 h | Yes | Cossarini-dunier et al. 1987[j,k,l] | Amphibian Immunity |
| Fish | *Cyprinus caprio* | Chemoluminescence of kidney and spleen macrophages phagocytosing opsonized *Yersinia ruckeri* | No effect | - | 7000-28000 | Technical | PE | 2 h | Yes | Cossarini-dunier et al. 1987[j,k,l] | Amphibian Immunity |
| Frog | *R. clamitans* | No. of *Echinostoma trivolvis* cercariae | Unknown, but a 53% increase at 15 µ/L | - | 15, 100 | Technical | PE | 10 h for tadpoles and cercariae | Yes | Griggs and Belden 2008[m,n] | Amphibian Parasite Infections |
| Frog | *R. sylvatica* | No. of *Echinostoma trivolvis* cercariae | Unknown | - | 15, 100 | Technical | PE | 10 h for tadpoles and cercariae | Yes | Griggs and Belden 2008[m,n] | Amphibian Parasite Infections |
| Frog | *R. sylvatica* | No. of *Echinostoma trivolvis* cercariae | Unknown | - | 15, 100 | Technical | PE | 14 d | Yes | Griggs and Belden 2008[m,o] | Amphibian Parasite Infections |
| Frog | *R. sylvatica* | No. of *Echinostoma trivolvis* cercariae | Increased when tadpole, but not cercariae, were exposed | 30 | 3, 30 | Commercial; Aatrex[i] | SR, PE | 31 d for tadpoles, 1h for cercariae | Yes | Koprivnikar et al. 2007[i,j,p] | Amphibian Parasite Infections |
| Frog | *Hyla versicolor* | No. of larval trematodes | Unknown | - | Unknown | Commercial | FS | Unknown | Yes | Koprivnikar et al. 2006[q] | Amphibian Parasite Infections |
| Frog | *R. pipiens* | No. of helminths | Unknown | - | Unknown | Commercial | FS | Unknown | Yes | King et al. 2007[r] | Amphibian Parasite Infections |
| *Frog* | *Rana clamitans* | Discontinuous gonads (abnormal segmentation) | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s] | Gonadal Morphology; Testes |
| *Frog* | *Rana clamitans* | Discontinuous gonads (abnormal segmentation) | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s] | Gonadal Morphology; Testes |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| *Frog* | *Rana clamitans* | Intersex (having testicular and ovarian tissues) | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s] | Gonadal Morphology; Testes |
| *Frog* | *Rana clamitans* | Size irregularities | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s] | Gonadal Morphology; Testes |
| *Frog* | *Rana clamitans* | Testicular ovarian follicles (testicular oocytes) | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Discontinuous gonads (abnormal segmentation) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Discontinuous gonads (abnormal segmentation) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM + 2-3 months | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Intersex (having testicular and ovarian tissues) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM + 2-3 months | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Mixed sex (single gonad has both ovarian and testicular tissue) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Mixed sex (single gonad has both ovarian and testicular tissue) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM + 2-3 months | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Other gonadal abnormalities | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Size irregularities | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Size irregularities | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM + 2-3 months | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Testicular ovarian follicles (testicular oocytes) | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM+ 2-3 months | Yes | Coady et al. 2005[s] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Discontinuous (multiple) testes or intersex | Reported an increase | 0.4, 0.8, 1.0, 10, 25, 200 | Exp. 1: 0.1, 1.0, 10, 25 and Exp 2: 0.1, 0.4, 0.8, 1.0, 25, 200 | Technical | SR | LTM | Yes | Hayes et al., 2002b[l,t] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Underdeveloped testes, closed or absent tubules, or low to absent germ cells | Reported an increase | 0.1, 25 | 0.1, 25 | Technical | SR | LTM | Yes | Hayes et al. 2003[l] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Underdeveloped testes, closed or absent tubules, or low to absent germ cells | Reported an increase | 0.8 | ND[p], 0.2, 0.3, 0.5, 0.8, 6.7 | Commercial | FS | Unknown | Yes | Hayes et al. 2003[l] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Testicular ovarian follicles (testicular oocytes) | Reported an increase | 0.1, 25 | 0.1, 25 | Technical | SR | LTM | Yes | Hayes et al. 2003[l] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Testicular ovarian follicles (testicular oocytes) | Reported an increase | 0.2, 0.3, 0.5, 0.8, 6.7 | ND[p], 0.2, 0.3, 0.5, 0.8, 6.7 | Commercial | FS | Unknown | Yes | Hayes et al. 2003[l] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Gonadal somatic index (testis size corrected for body size) | Unknown | - | <0.1-4.14 | Commercial | FS | Unknown | Yes | Hecker et al. 2004[s] | Gonadal Morphology; Testes |

Table S1 continued

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|---|---|---|---|---|---|---|---|---|---|---|---|
| *Frog* | *Xenopus laevis* | Discontinuous gonads (abnormal segmentation) | Unknown | - | 1, 10, 25 | Not reported | PE | Single exposure at beginning of study | Yes | Jooste et al. 2005[u] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Testicular ovarian follicles (testicular oocytes) | Unknown | - | 1, 10, 25 | Not reported | PE | Single exposure at beginning of study | Yes | Jooste et al. 2005[u] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Dilated tubules | Reported none | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | LTM | Yes | Kloas et al. 2009[v] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Gonadal area | Reported none | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | LTM | Yes | Kloas et al. 2009[v] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Mixed sex (single gonad has both ovarian and testicular tissue) | Reported none | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | LTM | Yes | Kloas et al. 2009[v] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Open (spacious) testicular lobules | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Open (spacious) testicular lobules | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Testes |
| *Frog* | *Rana pipiens* | Testicular ovarian follicles (testicular oocytes) | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Testicular ovarian follicles (testicular oocytes) | Unknown | - | 0.13-3.84 | Commercial | FS | Unknown | Yes | Smith et al. 2005[s] | Gonadal Morphology; Testes |
| *Fish* | *Carassius auratus* | Space between spermatogenic lobules | Reported an increase | 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Testicular resorption (atresia) | Reported an increase (70%) | 21 | 21 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002a[l] | Gonadal Morphology; Testes |
| *Frog* | *Xenopus laevis* | Testicular volume | Reported a reduction | 21 | 21 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002a[w] | Gonadal Morphology; Testes |
| *Fish* | *Carassius auratus* | Ovarian somatic index (ovary size corrected for body size) | Reported none | - | 100, 1000 | Analytical standard | PE | 30 and 56 days | Yes | Nadzialek et al. 2008[w] | Gonadal Morphology; Ovaries |
| *Fish* | *Carassius auratus* | Ovarian developmental stage | Reported none | - | 100, 1000 | Analytical standard | PE | 30 and 56 days | Yes | Nadzialek et al. 2008[w] | Gonadal Morphology; Ovaries |
| *Frog* | *Rana pipiens* | Percentage of immature follicles | Unknown | - | 10 atrazine and 10 atrazine + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Ovaries |
| *Frog* | *Rana pipiens* | Immature follicle diameter | Unknown | - | 10 atrazine and 10 atrazine + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Ovaries |
| *Frog* | *Rana pipiens* | Mature follicle diameter | Unknown | - | 10 atrazine and 10 atrazine + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Morphology; Ovaries |
| *Fish* | *Carassius auratus* | Proportion of oocytes undergoing atresia | Reported an increase (up to 25% of oocytes evaluated) | 100, 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Morphology; Ovaries |
| *Frog* | *Xenopus laevis* | Frequency of primary oogonia | Reported a decrease | 21 | 21 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002b[w] | Gonadal Morphology; Ovaries |
| *Frog* | *Xenopus laevis* | Frequency of secondary oogonia | Reported an increase | 21 | 21 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002b[w] | Gonadal Morphology; Ovaries |
| *Frog* | *Xenopus laevis* | Frequency of atritic oogonia | Reported an increase | 21 | 21 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002b[w] | Gonadal Morphology; Ovaries |
| *Frog* | *Rana clamitans* | Sex ratio | Unknown | - | Control: 0.07-0.25, treatments: 10, 25 | Technical | SR | 273 days during larval period | Yes | Coady et al. 2004[s,x] | Sex Ratio |

Table S1 continued

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Frog* | *Xenopus laevis* | Sex ratio | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s,x] | Sex Ratio |
| *Frog* | *Xenopus laevis* | Sex ratio | Unknown | - | Control: 0.1-0.26, treatments: 0.1, 1.0, 10, 25 | Technical | SR | LTM + 2-3 months | Yes | Coady et al. 2005[s,x] | Sex Ratio |
| *Frog* | *Rana pipiens* | Sex ratio | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u,y] | Sex Ratio |
| *Fish* | *Danio rerio* | Sex ratio | Reported female biased | 22, 220, 2200 | 22, 220, 2200 | Not reported | PE | 6 months | Yes | Suzawa and Ingraham 2008[z] | Sex Ratio |
| *Fish* | *Pimephales promelas* | Spermatogenic cells types | Reported a decrease | 5, 50 | 5 and 50 | Technical | SR | 21 days | Yes | Bringolf et al. 2004[l] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Dividing gonocytes | Reported none | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | LTM | Yes | Kloas et al. 2009[v] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Internal melanophores | Reported none | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | LTM | Yes | Kloas et al. 2009[v] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Rana pipiens* | Proportion of spermatogonia | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Rana pipiens* | Proportion of spermatids | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Rana pipiens* | Proportion of spermatocytes | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Rana pipiens* | Proportion of spermatids | Unknown | - | 10 and 10 + 10mg/L sodium nitrate | Technical | SR | LTM | Yes | Orton et al. 2006[u] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Proportion of spermatogenic cells types | Unknown | - | 0.13, 0.27, 0.47, 1.03, 3.3, 3.82, 3.84 | Commercial | FS | Unknown | Yes | Smith et al. 2005[s] | Gonadal Function; Testicular Cell Types |
| *Fish* | *Carassius auratus* | Proportion of spermatogenic cells types | Reported none | - | 100 and 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Spermatogonial cell nests | Reported a decrease | 21 | nominal 21, actual 18 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002a[w] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Nursing cells | Reported a decrease | 21 | nominal 21, actual 18 | Technical | PE | 48 hours during gonadal differentiation | Yes | Tavera-Mendoza et al. 2002a[w] | Gonadal Function; Testicular Cell Types |
| *Frog* | *Xenopus laevis* | Estradiol in males | Unknown | - | Control =0.1-0.26 and treatments 0.1, 1.0, 10, 25 | Technical | SR | LTM | Yes | Coady et al. 2005[s] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Carassius auratus* | 11-ketotestosterone juvenile female | Reported decrease | 1000 | 100, 1000 | Analytical standard | PE | 30 and 56 days | Yes | Nadzialek et al. 2008[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Oncorhynchus mykiss* | Testosterone | Reported none | - | 2, 200 µg/kg intraperitoneal injection | Technical | II | 6 days | Yes | Salaberria et al. 2009[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Oncorhynchus mykiss* | Estradiol | Reported none | - | 3, 200 µg/kg intraperitoneal injection | Technical | II | 6 days | Yes | Salaberria et al. 2009[w] | Gonadal Function; Sex Hormone Concentrations |

Table S1 continued

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Fish* | *Carassius auratus* | Testosterone in adult males | Reported decrease | 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Carassius auratus* | Testosterone in adult males | Reported decrease | 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Carassius auratus* | Estradiol to testosterone ratio in adult males | Reported increase | 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Carassius auratus* | Estradiol in adult males | Reported increase | 1000 | 100, 1000 | Analytical standard | PE | 21 days | Yes | Spanò et al. 2004[w] | Gonadal Function; Sex Hormone Concentrations |
| *Fish* | *Pimephales promelas* | Estradiol male | Unknown | - | 25, 250 | Technical | FT | 21 days | Yes | USEPA 2005[l] | Gonadal Function; Sex Hormone Concentrations |
| *Frog* | *Xenopus laevis* | Clutch size, offspring survival and time to metamorphosis | Unknown | - | 1, 10, 25 | Technical | PE | Single exposure during larval period then monthly from metamorphosis to 2 years of age. | Yes | Du Preez et al. 2008[u,aa] | Gonadal Function; Reproductive success |
| *Fish* | *Oncorhynchus mykiss* | Plasma vitellogenin | Reported an increase | 2, 200 µg/kg intra-peritoneal injection | 2, 200 µg/kg intraperitoneal injection | Technical | II | 6 days | Yes | Salaberria et al. 2009[w] | Vitellogenin |
| *Fish* | *Carassius auratus* | Plasma vitellogenin | Reported none | - | 1000 | Technical | PE | 21 days | Yes | Spanò et al. 2004[w] | Vitellogenin |
| *Frog* | *Xenopus laevis* | Gonadal aromatase activity | Reported none | - | <0.1-4.14 | Commercial | FS | Unknown | Yes | Hecker et al. 2004[s] | Aromatase |
| *Fish* | *Carassius auratus* | )varian aromatase activity and gene expression in juveniles | Reported none | - | 100, 1000 | Analytical standard | PE | 30 and 56 days | Yes | Nadzialek et al. 2008 | Aromatase |

[a] LTM = Early larvae to metamorphosis

[b] Detected substantial quantities of cyanazine, propazine, and simazine in stock solution made with well water indicating both contamination of stock solution and well water control

[c] Only a single egg mass, might not reflect general response

[d] Claim no significant effects but also provide $p<0.05$

[e] Provide no within-group variance estimate

[f] No data provided but conclude that there was no efffect of atrazine

[g] No statistics provided but conclude that there was no effect of atrazine

[h] No data were provided for each concentration and no degrees of freedom are provided for their statistical test

[i] Effects could be due to inactive ingredients

[j] No estimates of within-group variance anywhere in the manuscript raises questions about reported effects

[k] Conducted on only one fish and thus might not reflect a general response

[l] No statistics presented for these results

[m] Atrazine was a component of a mixture of pesticides tested and thus the experiment did not isolate the effects of atrazine

[n] Insufficient power to detect effects because they conducted infection when cercariae were moribund (after 10 h) and thus only had 5-10% infections; used 100 µ/L of ethanol as solvent control, which had significant effects on cercariae and peculiar effects on infections relative to water controls; 10 h exposure is unlikely enough time for atrazine to be absorbed/processed and to subsequently significantly alter immune cell production

[o] Did not control for mortality because they did not have reliable survival data; it is likely that the most infected individuals died confounding their results for parasite loads

[p] Did not control for atrazine-induced mortality prior to cercarial exposure, which has ben shown to be important by Rohr et al. 2008c

[q] Insufficient power to detect any effect of atrazine; only 1 of 12 ponds had levels of atrazine above the method detection limit

[r] Wetlands within each of their pesticide treatment categories were clustered spatially confounding the entire study

[s] Negative control or reference sites were contaminated with atrazine. Therefore, results of atrazine treatment relative to a contaminated control or reference are not valid.

[t] Data from each experiment were not reported separately, nor were means and variances for each treatment.

[u] Unusually high percentages of gonadal abnormalities in a control treatment. Hayes et al., (2002), Carr et al., (2003), Kloas et al., (2008), and Oka et al., (2008) all report <1% intersexes or testicular ovarian oocytes in controls. The negative control in this study was not functioning properly, so we can not draw conclusions by comparing atrazine treatments to control tanks.

[v] No test statistics, degrees of freedom, or means and variances are presented. Therefore, we can not evaluate statistical methods, results, or conclusions.

[w] Study is pseudoreplicated in that they inappropriately used individuals, rather than dosed tanks, as the replicates.

[x] Statistics were inappropriately conducted individually for each tank and not on treatment means. Thus, no treatment means or variance estimates are available.

[y] Reported female biased sex ratios associated with atrazine. However, more than 12% of control animals were intersexes which precluded our ability to determine if statistical significance was driven by differences in the numbers of females or intersexes.

[z] Statistical methodology, results (e.g. test statistics, degrees of freedom), and treatment means and variances are not reported.

[aa] The animals in this study were exposed during the Jooste et al. (2005) study where it is reported that more than 50% of individuals in controls had testicular ovarian follicles (see comment u).

[bb] FS = Field study, FT = Flow through experiment, II = Intraperitoneal injection, PE = Pulse experiment, SR = Static renewal experiment

Table S1 continued

**Table S2.** Summary of the effects of atrazine on sex ratio, vitellogenin, and aromatase. Excluded studies can be found in Table S1.

| Taxon | Species | Endpoint | Effect direction | Conc. where effect was observed (µ/L) | Conc. tested (µ/L) | Atrazine grade | Experiment type[a] | Exposure duration | Reference |
|---|---|---|---|---|---|---|---|---|---|
| **Sex ratio** | | | | | | | | | |
| Frog | *Xenopus laevis* | Sex ratio | None | - | 1.0, 10, 25 | Technical | SR | ~78 days during larval period | Carr et al. 2003[b] |
| Frog | *X. laevis* | Sex ratio | None[d] | - | ND[b], 0.12, 0.32, 0.68, 0.84, 1.23 | NA | FS | Unknown | Du Preez et al. 2005[c,d] |
| Frog | *X. laevis* | Sex ratio | None | - | 0.1, 1.0, 10, 25, 100 | Technical | SR | 75 days or less if metamorphosed earlier | Kloas et al. 2009[c] |
| Frog | *X. laevis* | Sex ratio | Female biased | 10, 100 | 0.1, 1, 10, and 100 | Technical | SR | ~65 days during larval period | Oka et al. 2008[c,e] |
| **Vitellogenin** | | | | | | | | | |
| Fish | *Pimephales promelas* | Plasma vitellogenin | None[f] | - | 5, 50 | Technical | SR | 21 days | Bringolf et al. 2004[c,f] |
| Fish | *Cyprinus carpio* | Liver vitellogenin gene expression | None | - | 0, 7, 35, 108, 277 | Unclear, presumably technical | PE | 0, 1, 4, and 30 days | Chang et al. 2005 |
| Frog | *Rana clamitans* | Plasma vitellogenin | None | - | ND-3.13 (see comments) | Commercial | FS | Unknown | McDaniel et al. 2008[c,g] |
| Frog | *R. pipiens* | Plasma vitellogenin | None | - | ND-3.13 (see comments) | Commercial | FS | Unknown | McDaniel et al. 2008[c,g] |
| Fish | *Danio rerio* | Whole embryo vitellogenin | None | - | 216, 1620 | Unclear, presumably technical | SR | 5 days | Muncke et al. 2007[c] |
| Fish | *P. promelas* | Plasma vitellogenin | None<br>None | - | 25, 250 | Technical | FT | 21 days | USEPA 2005 |
| **Aromatase** | | | None | | | | | | |
| Frog | *X. laevis* | Testicular aromatase gene expression | None | - | 1, 25, 250 | Technical | SR | 36 days | Hecker et al. 2005b[c] |
| Frog | *X. laevis* | Testicular aromatase gene expression | None | - | 1, 25, 250 | Technical | SR | 36 days | Hecker et al. 2005b[c] |
| Frog | *X. laevis* | Testicular aromatase gene expression | None | - | 10, 100 | Technical | SR | 49 days | Hecker et al. 2005a |
| Fish | *D. rerio* | Whole body aromatase activity of juveniles | None | - | 1, 10, 100 | Unclear, presumably technical | SR | 3 days | Kazaeto et al. 2004 |
| Frog | *R. clamitans* | Adult ovarian aromatase activity (Aug.-Sept. 2002) | Increased in agricultural areas | ag. sites ranged from ND-250 | ND-2[h] | Commercial | FS | Unknown | Murphy et al. 2006b[i] |
| Frog | *R. clamitans* | Juvenile ovarian aromatase (July 2002 and 2003) | Increased in agricultural areas | ag. sites ranged from ND-251 | ND-2[h] | Commercial | FS | Unknown | Murphy et al. 2006b[i] |
| Frog | *X. laevis* | Testicular aromatase gene expression | None | - | 1, 10, 25 | Technical | SR | ~65 days during larval period | Oka et al. 2008[j] |
| Fish | *D. rerio* | Gonadal aromatase (Cyp 19A1) gene expression | Increased | 2.2, 22, 220, 2200 | 2.2, 22, 220, 2200 | Unclear, presumably technical | PE | 3 days | Suzawana and Ingraham 2008 |

[a] FS = Field study, FT = Flow through, PE = Pulse experiment, SR = Static renewal experiment

[b]ND = Nondetectable

[c]No test statistics or degrees of freedom are presented. However, means and variances are presented.

[d]One water sample taken in late winter when runoff and fall out from precipitation would be minimal or non-existent is not conclusive evidence that atrazine was not present at the affected reference site during other times of the year.

[e]Lack of replication in the highest atrazine treatment due to high mortality in one of the two replicates, so conclusions about this specific treatment should be made with caution.

[f]Plasma vitellogenin was significantly higher in atrazine and solvent control treatments. Higher plasma vitellogenin in the atrazine treatment was, therefore, likely due to an effect of the solvent.

[g]Atrazine concentration for the non-agricultural reference site during 2003 is reported incorrectly. Repeated attempts to contact the author for clarification have not been forthcoming.

[h]Concentrations were between ND and 2 except on two occasion at one site when levels were 65 and 250 µ/L.

[i]Murphey et al. (2006b) dismiss these significant results because aromatase and atrazine concentrations (sampled four weeks prior to frog collection) do not correlate. However, no statistics are presented to support this claim, and atrazine concentrations sampled four weeks before frog sampling are not expected to correlate with gene expression and protein activity .

[j]There was no sexual dimorphism in aromatase expression at 0.1µg/L of atrazine apparently due to a (nonsignificant) decrease in female aromatase expression.