

Supporting Online Material

Methods

Table S1, S2

Literature Cited

Methods

We quantified the veracity of statements made in Solomon et al.'s (2008) review of the effects of atrazine on freshwater fish, amphibians and reptiles by carefully examining whether their representation of the primary literature was accurate. We documented statements in Solomon et al. (2008) that were inaccurate or misleading, explain why the statement is inaccurate or misleading, and provide the perceived effect that each of these statements would have for Syngenta, Inc., the company that produces atrazine and funded the Solomon et al. (2008) review (Table S1). We also quantified bias in Solomon et al.'s (2008) treatment of the primary literature by quantifying the proportion of studies criticized by Solomon et al. (2008) that did and did not find statistically significant effects of atrazine (at ecologically relevant concentrations) that could be perceived as adverse (Table S2).

Table S1. Inaccurate or misleading quotes from Solomon et al. (2008), the presumed effect the quote would have for Syngenta, the company who makes atrazine and funded the Solomon et al. (2008) review, and an explanation for why the quote is either inaccurate or misleading.

Count	Page in paper	Quote from Solomon et al. (2008)	Inaccuracy	Misleading statement	Effect statement would have for Syngenta ^a	Reference to which quote refers	Explanation for why the quote is inaccurate or misleading
1	727	"For amphibians, the smallest LC50 was reported to be 410 µg atrazine/L."	X		Positive	-	LC50s for several species in Storrs and Kiesecker (2004) were below 410 µg/L, as low as 3 µg/L killed nearly 100% of <i>Bufo americanus</i> after six days, whereas controls had nearly 100% survival.
2	727	"The most sensitive [nonlethal] endpoint reported [for amphibians]...was time to development in <i>X. laevis</i> with a LOEC of 100 µg/L."	X		Positive	-	Several studies have shown significant effects on nonlethal endpoints well below 100 µg/L, e.g. Kiesecker (2002) immunity and infections, Brodtkin et al. (2007) immune responses, Carr et al. (2003) abnormal swimming, Rohr and Palmer (2004) locomotor activity, Sullivan and Spence (2003) size at metamorphosis.
3	728	"It appears that charcoal filters were used in the tanks...Since charcoal will absorb atrazine, this may have affected exposure concentrations...this...may seriously have compromised the study."	X		Positive	Rohr et al. 2004	The authors state that they placed charcoal filtered water in the tanks and then dosed with atrazine. They provide actual concentrations that closely match nominal concentrations and even demonstrate that there was no significant atrazine degradation between weekly water changes. Filtration systems were not placed in tanks.
4	728-729	"The fact that different sources of atrazine were used in the two studies...raise serious questions about the analysis of the results and whether the reported effect were related to exposure or not."	X		Positive	Rohr et al. 2004	Technical grade atrazine was used each year of the experiment. What differed was the purity level between years (80 vs 99%). Out of 13 response variables, only one showed a year-by-atrazine interaction, indicating that responses between the two years were similar and that the differences in the purity of atrazine did not "raise serious questions about the analysis of the results and whether the reported effects were related to exposure or not".
5	728-729	"The fact that ...[actual] concentrations were not reported raise serious questions about the analysis of the results and whether the reported effect were related to exposure or not."	X		Positive	Rohr et al. 2004	Actual concentrations were reported in Rohr et al. (2004) on page 1035.
6	729	"Differences in the percent hatch, survival until d 16, day of hatch, percent larvae in refuge, mean day of metamorphosis, and snout-vent length at metamorphosis (author Table 1) were not large and are of questionable biological significance"		X	Positive	Rohr et al. 2004	An 11% reduction in hatching success, 13% decrease in embryo development, 8% reduction in refuge use, 3.2 day delay in metamorphosis are not necessarily small and biologically irrelevant responses, especially given that 3 days could mean the difference of desiccating or surviving in a drying pond.

7	729	"The interpretation of the responses by regression of transformed data ($\log x + 1$) was also in error since the control, with zero nominal concentration, cannot be made proportional to the other nominal concentrations and should only have been used as a reference point."	X	Positive	Rohr et al. 2006	There is nothing fundamentally wrong with using a zero concentration in a regression analysis. You would not want to use the zero concentration to calculate an LC50 value and the zero concentration should be used as a reference in this scenario. However, if one wants to test if there is a significant relationship between concentration and a response, it is completely valid to include the control concentration and to transform the data to improve fit. Hence, this was not in error.
8	731	"The authors reported 'counterintuitive' responses in that survivorship... was less (20%) at the nominal concentrations of 3 $\mu\text{g/L}$, compared to 50% at 30 and 100 $\mu\text{g/L}$ and 15% in the control."	X	Positive	Storrs and Kiesecker 2004	Survivorship in the controls in this study was 85% not 15%. Furthermore, a non-monotonic response is not necessarily "counterintuitive" given the many cases of non-monotonic dose responses with atrazine and other chemicals. It simply means that different mechanisms are functioning across the concentration gradient.
9	731	"Given the lack of concordance with observations in other studies, other factors such as the presence of unknown stressors and/or confounders are more likely explanations."	X	Positive	Storrs and Kiesecker 2004	Numerous studies on atrazine have revealed non-monotonic dose responses and thus this is not incongruent. The only factors that are known to differ systematically across the tanks were the treatments, so it is unlikely that "unknown stressors and/or confounders" could have caused these effects. Secondly, we are unaware of any natural stressors that could have generated these non-monotonic dose responses. However, it is possible and likely that the mortality associated with atrazine was caused by an indirect rather than direct effect. Atrazine certainly could have altered interactions between the tadpoles and opportunistically parasitic microbes in these tanks in complex, and perhaps non-linear, ways.
10	731	"Other laboratory studies...have not reported adverse effects on larval growth, developmental rate, mortality, time to metamorphosis, or size at metamorphosis in female or male frogs (...Carr et al. 2003...)"	X	Positive	Carr et al. 2003	Carr et al. (2003) reported that atrazine significantly reduced the proportion of animals reaching metamorphosis.
11	731	"Other laboratory studies...have not reported adverse effects on larval growth, developmental rate, mortality, time to metamorphosis, or size at metamorphosis in female or male frogs (...Coady et al. 2004...)"	X	Positive	Coady et al. 2004	Coady et al. (2004) reported significant decreases in both developmental rates and size at metamorphosis with exposure to 10 $\mu\text{g/L}$ of atrazine.
12	731	"Other laboratory studies...have not reported adverse effects on larval growth, developmental rate, mortality, time to metamorphosis, or size at metamorphosis in female or male frogs (...Coady et al. 2005...)"	X	Positive	Coady et al. 2005	No dose response data were provided and it appears as though they lumped all the atrazine concentrations and compared them to the controls, but no degrees of freedom for the ANOVA are provided to verify this. Thus, we cannot assume there was no effect.

13	731	"Other laboratory studies...have not reported adverse effects on larval growth, developmental rate, mortality, time to metamorphosis, or size at metamorphosis in female or male frogs (...Kloas et al. 2008...)"	X	Positive	Kloas et al. 2008	Kloas et al. (2008) reported significant decreases in size at metamorphosis with exposure to 0.01, 1, or 100 µg/L of atrazine.
14	731	"Effects reported in alligators...have not been linked to atrazine exposure."	X	Positive	-	This is not completely true. The following is a quote from Vonnier, Crain, McLachlan, Guillette and Arnold. 1996. EHP "Atrazine induced gonadal mesonephros aromatase activity in male hatchling alligators that was neither characteristic of male nor female."
15	731	"several key deficiencies in the experimental design of this study makes its interpretation difficult...It is not certain what the exposures to cercaria inside the enclosures were and if they were the same between sites."	X	Positive	Kiesecker 2002	The fact that cercarial abundance was not quantified in the enclosures does not make this study difficult to interpret because, within each pond, half the enclosures prevented cercariae of <i>Ribeiroia</i> sp. from entering, whereas the other half permitted their entrance. Hence, half the frogs were not exposed to the trematodes while the other half were. Agrochemicals can alter both exposure to trematodes and amphibian susceptibility to trematodes, and thus it is not necessary to ensure that there was equal exposure among the sites to assess whether ponds receiving agrochemicals have frogs with greater trematode-associated limb deformities. In addition, this work cannot be taken out of context from the laboratory experiment that showed that atrazine exposure increased <i>Ribeiroia</i> sp. trematode loads.
16	731	"several key deficiencies in the experimental design of this study makes its interpretation difficult...the lack of information on number of meta-cercarial cysts makes it difficult to know whether, in general, this was the result of greater infection rates or more infections in the limb buds of tadpoles at these sites."	X	Positive	Kiesecker 2002	The authors are correct that sites receiving atrazine might have had more infections at the limb buds than more total infections, but this is not supported by the follow-up laboratory experiment where more <i>Ribeiroia</i> sp. infections were found in frogs exposed to atrazine. It is also not supported by the experimental work of Rohr et al. (2008). Further, regardless of the reason, frogs at sites containing atrazine had more trematode-associated limb deformities. The exact reason for this field pattern was not explicitly elucidated by the field experiment (although it appears to have been elucidated by the lab study), but this is not a "key deficiency in the experimental design" that makes "its interpretation difficult."
17	732	"The temperatures of the ponds were not reported. Since temperature has an effect on amphibian development, this also may have confounded the comparisons between ponds."	X	Positive	Kiesecker 2002	Temperature does affect amphibian development, but there is no evidence that the temperatures experienced by these ponds could cause the limb deformities observed. Hence, there is no good reason to believe that this somehow confounded the results.

18	732	"Since only two concentrations were tested, a concentration-responses relationship could not be developed, thus limiting the interpretation of the results."	X	Positive	Kiesecker 2002	A concentration-response relationship, and whether this response is non-linear, can be established with three concentrations, which is the number Kiesecker had (0, 3, 30). You cannot calculate an accurate LC50 with three concentrations, but you can assess non-linearity and significance of a dose response. Even if only two concentrations were chosen, it would not limit the interpretation relative to any other study. In any study, conclusions should only be drawn within the concentration range chosen unless concentration is treated as a random variable or if proper adjustments to the confidence intervals are made beyond the concentration range tested. Kiesecker drew appropriate conclusions about the concentrations he tested.
19	732	"Toxicity values for cercaria of several other species of trematode were also reported and 12-h LC50s ranged from <20 µg/L for <i>Haematoloechus</i> sp., 92 µg/L for <i>Alaria</i> sp., 110 µg/L for <i>E. trivolvis</i> , to >850 µg/L for <i>Megalodiscus</i> sp. (Koprivnikar et al. 2006)."	X	Neutral	Koprivnikar et al. 2006	Koprivnikar et al. 2006 miscalculated their LC50s in two different ways. First of all, they used a probit value of 0.5 which does not equate to 50% mortality. Second, they did not control for the mortality in the control treatment. This latter error results in a substantial underestimate of the LC50 values because there was indeed mortality in all the controls. If one does not control for mortality in the control group and a control group has 50% mortality, then the estimated LC50 value for the chemical would be 0 µg/L. Thus, the LC50 cannot be calculated for any of the trematodes in this study. Solomon et al. (2008) criticized Rohr et al. (2006) for inappropriately for using the control treatment in a regression analysis (even though it was not inappropriate) but did not criticize Koprivnikar et al. (2006) for using the control treatment to calculate an LC50. At least two requests were made by Rohr to fix this error, but Koprivnikar chose not to submit a correction.
20	732	"Given the relatively small number of tadpoles per replicate (21 per treatment), these results are difficult to interpret"	X	Positive	Koprivnikar et al. 2007	There is nothing wrong with having a single animal per replicate and testing 21 animals per treatment. In fact, a sample size of 21 per treatment is greater than most studies in Solomon et al.'s (2008) review. Additional animals per replicate increase the precision of the estimate per replicate but if this comes at the cost of reducing the total number of replicates, then this would reduce sample size and statistical power. While there are other concerns with this experiment, a single animal per replicate with 21 replicates per treatment does not make the experiment or results "difficult to interpret".
21	732	"Under most conditions of exposure in the field, atrazine and its formulants would be expected to not co-occur."	X	Positive	Koprivnikar et al. 2007	While Syngenta and the authors might have this information, the inactive ingredients are proprietary and thus there is no publicly available scientific evidence to support this claim of which we are aware.

22	732	"the results of the study do not appear to be internally consistent or reproducible and are not ecologically relevant."	X	Positive	Koprivnikar et al. 2007	There is nothing inconsistent regarding the results of this study. One would not necessarily expect the same results (on infection abundance) if only the parasite was exposed to atrazine, if only the tadpole was exposed to atrazine, or if both were exposed to atrazine. No justification for this claim is provided.
23	732-733	"A mixture of 5 µg/L atrazine and 5 µg/L carbaryl did not affect the incidence of embryonic deformities, hatching success, survival to metamorphosis, tadpole mass, bony triangles, skin webbing, or multiple deformities."	X	Positive	Bridges et al. 2004	Bridges et al. (2004) showed that the mixture of atrazine and carbaryl significantly reduced survival to metamorphosis (Bridges et al. 2004, Figure 4).
24	732-733	"A mixture of 5 µg/L atrazine and 5 µg/L carbaryl did not affect the incidence of embryonic deformities, hatching success, survival to metamorphosis, tadpole mass, bony triangles, skin webbing, or multiple deformities."	X	Positive	Bridges et al. 2004	Bridges et al. (2004) showed that the mixture of atrazine and carbaryl significantly increased the proportion of metamorphs with skin webbing deformities (Bridges et al. 2004, Figure 6B).
25	732-733	"A mixture of 5 µg/L atrazine and 5 µg/L carbaryl did not affect the incidence of embryonic deformities, hatching success, survival to metamorphosis, tadpole mass, bony triangles, skin webbing, or multiple deformities."	X	Positive	Bridges et al. 2004	Bridges et al. (2004) showed that the mixture of atrazine and carbaryl significantly increased the proportion of metamorphs with multiple deformities (Bridges et al. 2004, Figure 6C).
26	733	"Extracts from water from a reference pond in Minnesota were associated with a small incidence (5%) of deformities, indicating that other environmental contaminants [not atrazine] or factors are potentially responsible for the observed abnormalities noted under field conditions (Bridges et al. 2004)."	X	Positive	Bridges et al. 2004	Of the frogs exposed to water from the reference site, 0% had bony triangle deformities, approximately 40% had skin webbing deformities, and approximately 35% had multiple deformities compared to approximately 17%, 65%, and 45%, respectively, of the animals exposed to the mixture of atrazine and carbaryl. These values are no where near 5%. No deformities were found in frogs exposed to the well water alone. Bridges et al. (2004) provide no information about this "reference site" other than annual surveys exhibited low levels of deformities. However, the authors also note that most deformed animals are not expected to live long in nature, which would result in underestimates of deformities in the field. No quantification of chemicals in the water from the "reference site" was provided.

27	733	"Overall, there is no credible evidence to suggest that atrazine directly causes or contributes to limb deformities caused by parasites in frogs."	X	Positive	-	See Bridges et al. (2004) and Kiesecker (2002).
28	734	"No effects on sex ratios in mammals were reported in multigenerational studies (U.S. EPA 2000, Gammon et al. 2005)."	X	Positive	Gammon et al. 2005	An electronic search of this paper using "ratio", "sex" and "sex ratio" reveals that mammalian sex ratios are not discussed in this manuscript. In fact, only one reference to sex ratio is discussed and it focuses on anurans.
29	734	"Given the fact that weights were determined to the nearest gram, the small differences were close to the limits of detection and raise questions about possible measurement errors."	X	Positive	Beldomenico et al. 2007	By definition, a statistically significant difference in weight, reported by Beldomenico et al. (2007), was detectable. In addition, if weight was measured at a more precise scale the power to detect differences would have been higher. Therefore, in opposition to what Solomon et al. (2008) imply, finding differences at such a coarse scale where power to detect effects is lower is conservative.
30	734	"In addition, the physiological significance of these differences in weight was not tested in this study."	X	Positive	Beldomenico et al. 2007	This statement implies that not knowing the physiological significance somehow negates the effect.
31	734	Other studies on eggs of red-eared slider turtle (<i>Pseudemys elegans</i>) and <i>A. mississippiensis</i> showed no response in terms of sex ratio to nominal aqueous exposures of as great as 500 µg atrazine/L used to drench the eggs (Gross, 1999a, 1999b)"	X	Positive	Gross et al. 1999a,b	Gross et al. (1999a,b) are not peer reviewed documents and therefore cannot be substantiated.
32	734	Laboratory studies on <i>R. clamitans</i> exposed to atrazine at concentrations as great as 25 µg/L (Coady et al. 2004) and <i>X. laevis</i> at concentrations as great as 100µg/L (Coady et al. 2005; Kloas et al. 2008) did not reveal any effects on sex ratio.	X	Positive	Coady et al. 2004, 2005	Coady et al. (2004, 2005) have contaminated negative controls. Therefore, any non-significant effects of atrazine relative to the contaminated control are not legitimate. Many of the same authors from Solomon et al. (2008) are co-authors on the Coady et al. (2004, 2005) papers, so they were aware of the illegitimate comparisons.
33	734	No effects of sex ratio were observed in NF stage 66 metamorphs in studies on <i>X. laevis</i> exposed to concentrations of atrazine as high as 30 µg/L in outdoor mesocosms (Jooste et al. 2005)	X	Positive	Jooste et al. 2005	No sex ratios are reported in this study. However, the data were available and a chi square analysis shows that, at 10 µg/L, the population was female biased (df=1, $\chi^2 = 6.82$ p<0.01). This finding is consistent with other studies (Orton et al 2006, Oka et al. 2008).

34	734	"If atrazine were to affect maturation of the gonads, this would be manifested in terms of a quantitative difference in the distribution of cell types within the testis and ovary, through effects on sperm production, or fecundity. There is no evidence to support the contention that atrazine affects sexual development in fish or amphibians."	X	Positive	-	Maturation of the gonad or sexual maturation is a distinct phenomenon from sexual development which involves gonadal development during embryogenesis. Therefore, this statement is not logical. In fact, atrazine can influence both gonadal maturation (e.g. spermatogenesis) (USEPA, 2005; Orton et al 2006; Tavera-Mendoza et al. 2002a) and gonadal development (e.g. ambiguous gonads, discontinuous gonads, multiple gonads, mixed sex gonads etc.) (Carr et al 2003; Hayes et al. 2003).
35	735	"No differences in the absolute or relative number of testicular cell types were observed in <i>X. laevis</i> from corn and non-corn growing areas in South Africa where atrazine concentrations ranged from 0-9µg/L"	X	Positive	Smith et al. 2005	Reference sites in this study were contaminated (site means range from 0.13-0.47 µg/L) at concentrations that have induced effects in <i>X. laevis</i> in other studies (e.g., Hayes et al. 2002). Non-significant results of atrazine exposure relative to a contaminated reference site (reported by the authors, which include many co-authors from Solomon et al. 2008) are not valid. In fact, only one site during one collection time (1 of 6 measurements) had non-detectable atrazine concentrations. A regression approach might have been appropriate.
36	735	"In a study on <i>R. pipiens</i> from areas associated with row crop agriculture (and where atrazine and other pesticides were found), no differences were detected in the gonadal somatic indices or stage or spermatogenesis between frogs from agricultural and nonagricultural regions ($p > .05$) (McDaniel et al. 2008)"	X	Positive	McDaniel et al. 2008	Significantly more <i>R. pipiens</i> juveniles at the agricultural sites with highest mean atrazine concentrations were at a "more advanced stage of spermatogenesis than at other sites, with significantly more juvenile males at Stage III" (McDaniel et al. 2008).
37	735	"Orton et al. (2006) reported no difference in the total number of spermatogenic cells. However, they did report an increase in the percentage of testicular cells in the latter stages of spermatogenesis relative to controls (38% vs. 20%). The biological significance of this effect is unclear since there were no other effects of atrazine on testicular development, and only one atrazine concentration was tested."	X	Positive	Orton et al. 2006	Solomon et al. (2008) continually dismiss significant findings because their biological relevance is unknown. Our lack of understanding of the biological relevance of specific effects does not negate the significant effects. In fact, other effects on testicular development did occur. Sex ratios were significantly female biased at 10µg/L of atrazine when combined with 10mg/L of nitrate, suggesting that gonadal development was altered. Further, the proportion of spermatogonia (early stages of spermatogenesis) was higher in the atrazine only treatment whereas the proportion of spermatocytes (mid-spermatogenesis) was lower. Therefore, although the total number of germ cells was not significantly different among treatments, the proportion of each cell type and the process of sperm production were significantly affected by atrazine exposure.

38	735	"Responses at greater concentrations may have been confounded by general necrosis observed in several tissues of exposed tadpoles (Tavera-Mendoza, 2001).	X	Positive	Tavera-Mendoza et al. 2002a,b	The fact that atrazine induces necrosis across many tissues does not negate the fact that gonadal necrosis (e.g., testicular resorption and ovarian atresia) was significantly higher in atrazine-treated versus control frogs.
39	735	"The cause of necrosis was not clear."	X	Positive	Tavera-Mendoza et al. 2002a, b	If there is a treatment effect in a controlled experiment we must assume that the cause of a significant effect was the treatment, in this case atrazine. There is a 5% chance (or less) that atrazine did not induce the increased necrosis ($\alpha=0.05$).
40	736	"Furthermore, the responses reported in the testes were inconsistent with those reported elsewhere (Hayes et al. 2001; Coady et al. 2005; Carr et al. 2003)."	X	Positive	Tavera-Mendoza et al. 2002a	<i>X. laevis</i> tadpoles were only exposed for 48 h during sexual differentiation to atrazine in the Tavera-Mendoza (2002a) study, whereas Hayes et al. (2001), Coady et al. (2005), and Carr et al. (2003) exposed tadpoles until metamorphosis. In addition, Tavera-Mendoza (2002a) measured different endpoints. Therefore, responses reported in Tavera-Mendoza (2002a) are not expected to be identical to those in the other studies.
41	736	"The biological significance of the effects reported by Carr et al. (2003) remains unclear since mixed ovarian/testicular tissue was not observed at the histological level."	X	Positive	Carr et al. 2003	Carr et al. (2003) found that frogs raised in 25 ppb atrazine had ambiguous gonads (having characteristics of both males and females and thus were characterized as "intersexes") at metamorphosis, a time when gonads are typically clearly sexually dimorphic. The fact that subsequent histological analyses showed no intersex tissue (male and female germ cells) does not negate the fact that atrazine, at the ecologically relevant concentration of 25 ppb, induced altered gonadal development such that it was impossible to determine the sex of animals that should have been sexually dimorphic. The fact that we do not know the biological relevance for this and other abnormalities reported by Carr et al. (2003) (e.g., atrazine dose dependent increase in discontinuous gonads) does not negate the fact that atrazine induced abnormal gonadal development in this study.
42	737	"There was no apparent relationship between atrazine concentration and the incidence of TOFs..."	X	Positive	Coady et al. 2005	Again the negative control (0 ppb atrazine treatment) was contaminated with atrazine in the Coady et al. (2005) study. Therefore, non-significant results of atrazine treatments relative to a contaminated control are not valid.

43	737	"The observation of TOFs by Coady et al. (2005) is interesting given previous reports of this phenomenon by Gallien (1974) and recent data from microcosm studies in which <i>X. laevis</i> exposed to atrazine or control medium during larval development exhibited TOFs regardless of whether they were exposed to atrazine or not (Jooste et al. 2005)."	X	Positive	-	Jooste et al. (2005) found that control treatments had a high frequency of gross gonadal abnormalities (e.g. 8.8% of control male frogs had gross gonadal developmental abnormalities such as multiple testes). This frequency of abnormalities is uncommon (e.g. Hayes et al. 2002, 2003, Carr et al. 2003). Given that TOFs were found in conjunction with high incidences of gonadal abnormalities (e.g. three testes), which are not considered normal, we think the data of Jooste et al. (2005) should not be used as evidence for naturally occurring TOFs.
44	737	"They reported the presence of TOFs in males from the 0.1-and 25 µg atrazine/L treatment groups but not in the controls. This observation is confusing because it is also reported that two control animals with TOFs were observed (Hayes et al. 2003, p. 570)."	X	Positive	Hayes et al. 2003	Hayes et al. (2003) clearly distinguishes between oocytes found within the testis (referred to as TOFs by Solomon et al. 2008) and extragonadal oocytes, which are oocytes not incorporated within testicular tissue (found in two control males in Hayes et al. 2003).
45	737	"There was no atrazine-treated effects on incidence of intersex or phenotypic sex ratio (based on gonadal appearance)..."	X	Positive	Coady et al. 2004	Again the negative control (0 ppb atrazine treatment) was contaminated with atrazine in the Coady et al. (2004) study. Therefore, non-significant results of atrazine treatments relative to a contaminated control are not valid.
46	737	"Hayes et al. (2003) reported that only frogs collected from sites with measurable atrazine concentrations exhibited TOFs that were similar to those observed in the laboratory after exposure to atrazine. This was interpreted as suggesting that hermaphroditism or "sex reversal" never occurs in the absence of atrazine and that the phenomenon is solely the result of exposure to atrazine."	X	Positive	Hayes et al. 2003	The statement that Hayes et al. (2003) interpret their findings as never occurring in the absence of atrazine is untrue. In fact, the following quote is taken from the discussion section of Hayes et al. (2003, p. 572) "Atrazine may not be the only compound that induces testicular oogenesis, however. There may be many chemicals, natural products, and even populations that naturally display this phenomenon (Witschi 1929). Nevertheless, the present study showed that atrazine induced testicular oogenesis and hermaphroditism in a population that does not show this developmental pattern under controlled laboratory conditions, and that hermaphroditism in wild <i>R. pipiens</i> is associated with atrazine use and contamination."

47	738	"In addition, the study design (Hayes et al. 2003) was flawed because concentrations of atrazine were only measured at the time of collection of sexually differentiated frogs and there is no knowledge of their exposure when they were undergoing sexual differentiation prior to the time of collection. Since concentrations are not known during critical developmental stages, any conclusions reported by Hayes et al. (2003) are speculative."	X	Positive	Hayes et al. 2004	Having a more detailed understanding of the distribution of atrazine concentrations over time at each site studied in Hayes et al. (2003) would undoubtedly be ideal. Very few atrazine studies have ever achieved this goal since accurate atrazine analyses can be prohibitively expensive. Indeed, field studies conducted by Solomon and colleagues (e.g. Smith et al. 2005) have not analyzed atrazine concentrations on an extremely fine scale either. The fact that more analyses were not conducted does not negate the fact that gonadal abnormalities were found at every site where atrazine occurred at measurable levels. Finally, this study was not designed to explicitly link atrazine exposure during sexual differentiation to gonadal deformities. This type of analysis is more appropriately addressed through laboratory studies. Field studies are generally designed to determine if there is an association between atrazine exposure and gonadal abnormalities, which is what Hayes et al. (2003) argue they show.
48	738	"There was no correlation between the proportion of <i>R. pipiens</i> with TOFs and measured atrazine concentrations..."	X	Positive	McDaniel et al. 2008	McDaniel et al. (2008) actually report that "Although the proportion of testicular oocytes did not correlate directly with atrazine concentrations it did correlate with a mixture of pesticides and nutrients, particularly atrazine and nitrate..." In fact, the most important univariate component of their multivariate analysis investigating the factors associated with TOFs in <i>R. pipiens</i> was atrazine (see p. 237). In addition, <i>R. pipiens</i> with TOFs occurred significantly more often at agricultural sites where atrazine was highest (not a causal link but an association). Interestingly, TOFs did not differ between agricultural and non-agricultural sites for <i>R. clamitans</i> .
49	738	"Atrazine was present in greater concentrations in the agricultural sites than in the reference sites; however, several other pesticides were also detected in agricultural locations. Thus, causality could not be assigned."	X	Positive	McDaniel et al. 2008	Field surveys are not designed to test for causality. They test for association.

50	738	"In addition, studies of frog populations conducted after the introduction of atrazine have not found a relationship between the exposures of frogs to atrazine and the incidence of TOFs (Smith et al. 2005, Coady et al. 2005, Jooste et al. 2005, McDaniel et al. 2008, Smith, 2007 personal communication)."	X	Positive	Various	Smith et al. (2005) and Coady et al. (2005) have atrazine-contaminated reference sites and atrazine-contaminated controls; thus non-significant difference between the "controls" and atrazine sites are difficult to interpret. McDaniel et al. (2008) did find a relationship between TOFs and atrazine in a multivariate analysis that incorporated other environmental factors, such as nitrate and the number of other pesticides present (see above #48). Jooste et al. (2005) found gonadal abnormalities were common in all treatments including controls (e.g. 8.8% of control male frogs had gross gonadal developmental abnormalities such as multiple testes). This frequency of abnormalities is uncommon (e.g. Hayes et al. 2002, 2003, Carr et al. 2003), so the non-significant effect of atrazine on the incidence of TOFs is unclear (see above #43). Finally, the Smith 2007 personal communication cannot be evaluated. Taken together, these studies do not form a solid argument demonstrating that atrazine is not associated with TOFs.
51	738	"Testicular Ovarian Follicles in Frogs as a Natural Phenomenon."	X	Positive	Du Preez et al. (2008a) -- unpublished manuscript	In this section, Solomon et al. (2008) use data from an unpublished article (that can not be evaluated) to bolster their argument that TOFs are a normal phenomenon in <i>X. laevis</i> and to speculate that haplotype might explain the variable results (no atrazine induced TOFs and atrazine induced TOFs) across studies. As far as we can tell, Dupreez et al (2008a) have confounded genetic similarity (haplotype) and location. These two variables are, admittedly, very difficult to tease apart. However, because many pollutants known to induce reproductive developmental abnormalities are transported atmospherically and deposited in "pristine areas" (van Dijk 1999), we must determine that the locations where DuPreez et al. (2008a) collected <i>X. laevis</i> were endocrine disruptor free (not just atrazine free) before we can confidently argue that TOFs are natural in <i>X. laevis</i> .

52	738	"In a review of unexposed control Japanese medaka (<i>Oryzias latipes</i>)...TOFs were observed in 30% of all studies but with large variation between the four laboratories in the study (0-100%) (Grim et al. 2007). These observations suggest that as in frogs, the presence of TOFs in <i>O. latipes</i> is a natural phenomenon and that there may be variation between strains of fish."	X	Positive	Grim et al. 2007	TOFs were actually observed in 15 of 41 (37%) studies. Grim et al. (2007) warn against making the conclusion that Solomon et al. (2008) make here. For example Grim et al. (2007) argue "It is not our intent to report a universal prevalence figure for intersex in medaka, and we have deliberately avoided stating this type of conclusion for several reasons....our data lacked potentially useful ancillary information, such as chemical analysis of the food and water for EAS [endocrine active substances]." In fact, they go farther to suggest that "Such subpopulations [with TOFs] might correspond to particular sources of medaka. Although still feasible, this source hypothesis was not substantiated by the results of the present review: The GCRL and U.S. EPA shared the same original source of medaka; however, experiments performed at these two laboratories generated widely disparate prevalences of TO (Table 2)." Food and water sources, however, did vary between studies both of which can be contaminated with EAS (Grim et al. 2007).
53	739	"Differences in the numbers of ovarian melanophores (pigmented cells) between control and treated <i>X. laevis</i> were observed in only one of two laboratories in the recent study reported by Kloas et al. (2008)	X	Positive	Kloas et al. 2008	The in press Kloas et al. (2008) article in Toxicological Sciences that we found did not report data on ovarian melanophores. However, if our version is not the up to date manuscript and Solomon et al (2008) are correct, then ovarian melanophore numbers have been shown to be affected in two of three studies (Hayes et al. 2006 and one of the two studies conducted by Kloas et al. 2008).
54	739	"While effects on reproduction would represent a significant concern, the weight of the available evidence does not substantiate claims that atrazine is a reproductive toxicant that feminizes and demasculinizes male frogs."	X	Positive	Multiple	Although Solomon et al. (2008) attempt to negate or dismiss all reproductive effects induced by atrazine, industry funded, non-industry funded, and EPA studies report significant reproductive effects of atrazine or associations between atrazine exposure and gonadal abnormalities, such as multiple gonads, ambiguous gonads, mixed sex and intersex frogs, and altered spermatogenesis, gonadal size and shape, and testicular lobule characteristics.
55	739	"Based on the available information the only evidence for effects of atrazine on concentrations of steroid hormones in blood plasma are those reported by Hayes et al. (2002)."	X	Positive	Hayes et al. 2002	A critical evaluation of the articles cited by Solomon et al. (2008) revealed that 8 of 10 studies (80%), across diverse taxa (fish, amphibians, and rats), show a direct effect or an association between altered hormone levels and increased atrazine concentration (Cooper et al. 2000; Hayes et al. 2002; Spanò et al 2004; Hecker et al. 2004; Coady et al. 2005; Hecker et al 2005b; Murphy et al 2006a; McDaniel et al. 2008). Interestingly, three of these studies dismiss their own significant findings.
56	740	"Transcription of aromatase (CYP19 A1 and A2) in juvenile zebrafish was not significantly affected by exposure to concentrations as great as 1000 µg atrazine/L (Figure 8)."	X	Positive	Kazeto et al. 2004	The highest concentration that Kazeto et al. (2004) used was 100 ppb, not 1000 ppb.

57	740	"The report of increased expression of CYP19A1 in ovaries of <i>D. rerio</i> (Suzawa and Ingraham, 2008) is in contrast to the findings of Kazeato et al. (2004) but may have been the result of experimental design."	X	Positive	Suzawa et al. 2008	Solomon et al. (2008) go on to criticize aspects of the Suzawa et al.(2008) study. However, a very important point that is erroneously neglected is that the Kazeto et al. (2004) study used lower concentrations than Suzawa et al. (2008). The highest concentration used by Kazeto et al. (2004) was 100 ppb (or 460 nM), whereas the lowest concentration used by Suzawa et al. (2008) was 216ppb (or 1uM). Therefore, the findings of Suzawa et al. (2008) are not in contrast to Kazeto et al. (2004).
58	740	"There was no replication per treatment (one tank of 15 fish only per concentration), concentrations were not measured, and pooling of samples (5 fish each) for analysis may have obscured interindividual variability."	X	Positive	Suzawa et al. 2008	Each treatment in this study was replicated two times (Ingraham, personal communication). Calculating a tank mean (from 5 fish) is not inappropriate. In fact, treating animals within a tank as individuals would be pseudoreplication and thus inappropriate. Each fish would have had to be maintained separately in order to correctly calculate inter-individual variation. Although this is an interesting question, the point of Suzawa et al. (2008) was not to assess how individuals respond to atrazine but was to test if and how treatment means varied.
59	741	"As discussed elsewhere in this section, environmentally relevant concentrations do not induce aromatase in vivo in frogs or fish, which suggests that the response observed in mutated cells in vitro should not be extrapolated to whole organisms in the field. This also suggests that the extrapolations to human cancers discussed in the paper (Fan et al. 2007) are highly speculative at best and are not supported by the greater weight of evidence in the literature."	X	Positive	Fan et al. 2008	Fan et al. (2008) investigates the mechanisms through which aromatase is induced in human cancer cells. The idea that we should disregard research focused on determining the ways in which atrazine might be involved in human cancer pathogenesis because ecologically relevant concentrations have not been shown to increase aromatase in frogs and fish seems unusual.
60	742	"To support their findings, Hayes et al. (2006b) presented a photograph of gross morphology and micrographs, which was already published in an earlier manuscript. Author Figure 5 in Hayes et al. (2006b) states that the exposure concentration was 0.1 µg/L (0.1ppb), but the identical photographs also appeared in author Figure 2 in Hayes et al. (2002) where the text and caption state that the exposure was 1 µg/L (1ppb). The reason for the use of this figure and difference in treatment concentration is unclear."	X	Positive	Hayes et al. 2002, 2006	The use of this picture in both manuscripts is clear upon reading Hayes et al. (2006), which compares gonadal abnormalities induced by atrazine in Hayes et al. (2002) to those induced by the anti-androgen cyproterone acetate (CPA) and 17β-estradiol. The difference in the treatment concentration cited between publications must be a typo. We must assume that typos, such as this, and others (e.g. Solomon et al. (2008) stating that Kazeto et al. (2004) used atrazine treatments as high as 1000 µg/L when the highest concentration was only 100 µg/L--#56 herein) are honest mistakes unless we have credible evidence to the contrary.

61	742	"Interpreting the effects of chemicals, such as atrazine, on plasma hormones in frogs is difficult due to the relatively great degree of variation among individuals."	X	Positive	-	The meaning of this statement is unclear. Finding significant effects is certainly more difficult when a system is highly variable. However, when significant effects are found, they are no more difficult to interpret than effects measured in invariant systems. Indeed, the biological significance of findings is not always clear, but this does not negate the significant effect.
62	742	"Only one study has reported a change in testosterone in response to exposure to atrazine at environmentally realistic concentrations (Hayes et al. 2002)"	X	Positive	Hayes et al. 2002	In addition to the Hayes et al. (2002) study, several field studies have found an association between increased atrazine and altered testosterone concentrations. For example, testosterone concentrations were significantly negatively correlated with atrazine concentrations in <i>Xenopus laevis</i> (Hecker et al. 2004). Testosterone was also negatively correlated with atrazine in juvenile male and adult female <i>R. pipiens</i> , and 11-ketotestosterone was negatively correlated with atrazine in juvenile <i>R. pipiens</i> (McDaniel et al. 2008; author's do attempt to disregard this significant effect even though it occurs across sexes). Murphy et al. (2006b) show that testosterone is significantly higher in adult males and juveniles at agricultural sites than non-agricultural sites. Interestingly, estradiol to testosterone ratios were also higher in adult and juvenile males at agricultural sites, so increased testosterone could have been a physiological attempt to balance the relative concentrations of these hormones.
63	742	"A study in which juvenile <i>X. laevis</i> were exposed to a range of atrazine concentrations (0.1-25 µg/L) from 72h post hatch until 2-3 months post metamorphosis found no statistically significant differences in plasma testosterone concentrations among atrazine treatments and the controls (Coady et al. 2005)."	X	Positive	Coady et al. 2005	Again, the negative control (0 ppb atrazine treatment) was contaminated with atrazine in the Coady et al. (2005) study. Therefore, non-significant results of atrazine treatments relative to a contaminated control are not valid.
64	742	"No studies, conducted under controlled conditions, have been able to repeat the observation of a decrease in plasma concentrations of testosterone in male <i>X. laevis</i> at environmentally realistic exposure concentrations in the laboratory (Coady et al. 2005; Hecker et al. 2005) or the field (Hecker et al. 2004)."	X	Positive	Coady et al. 2005	Coady et al. (2005) has contaminated negative controls. Hecker et al. (2005) indeed found no effect of atrazine on testosterone. Several studies have found negative correlations between atrazine exposure in the field and testosterone concentrations (Hecker et al. 2004; McDaniel et al. 2008) or altered sex hormone concentrations in agricultural vs. non-agricultural areas (Murphy et al. 2006b; McCoy et al. 2008).

65	742	"It was reported that atrazine exposure reduced the concentration of testosterone in male <i>X. laevis</i> (Hecker et al. 2005a), but the lowest-observed effect concentration for this response was 250 µg atrazine/L."	X	Positive	Hecker et al. 2005b	Hecker et al. (2005a) is not the correct citation. Hecker et al. (2005b), however, does report a significant effect of atrazine on testosterone concentrations. In addition, this quote is in direct opposition to the quote above (#64). Indeed, Solomon et al. (2008) are implying that 250 µg/L is not ecologically relevant, but they have found this concentration on rare occasions in their own studies (e.g., Murphy et al 2006b) and several field studies have detected atrazine well above this concentration (reviewed in Rohr and McCoy 2010). Of the controlled experiments on <i>Xenopus laevis</i> conducted thus far using ecologically realistic concentrations of atrazine, which do not have <i>obviously</i> contaminated controls, two (Hayes et al. 2002; Hecker et al. 2005b) show that atrazine causes reductions in testosterone concentrations and one study does not (Hecker et al. 2005a).
66	743	"...the only study that claims an effect of atrazine at environmentally realistic concentrations is that of Hayes."	X	Positive	Hayes et al. 2002	See comment (#55) above. This comment is made repeatedly in Solomon et al. (2008) and is inaccurate.
67	743	"A negative correlation was found between concentrations of atrazine in water and concentrations of testosterone in plasma of adult female <i>X. laevis</i> inhabiting atrazine-exposed ponds in South Africa; however, due to the presence of other confounding factors, such as other agricultural chemicals, it was impossible to establish a direct cause-effect relationship between atrazine and plasma testosterone concentrations."	X	Positive	?	Correlations never imply causation and correlative field surveys are not typically designed to determine cause-and-effect relationships. Thus, dismissing these findings is odd. Finding a significant correlation between reduced testosterone and increased atrazine suggests that atrazine is associated with reduced testosterone and supports the hypothesis that atrazine is an endocrine disrupting chemical. This hypothesis can now be tested explicitly under a controlled setting.
68	743	"Concentrations of testosterone and 11-ketotestosterone in plasma of <i>R. clamitans</i> and <i>R. pipiens</i> from wetland areas in Ontario showed no correlation with concentrations of atrazine."	X	Positive	McDaniel et al. 2008	The abstract of McDaniel et al. (2008) reports that there were no effects of atrazine on sex hormone concentrations. The results, however, report that in one of three years studied, juvenile males at agricultural sites had significantly reduced testosterone, which was significantly negatively correlated with atrazine concentration. This is dismissed by the authors as being an artifact of a small sample size (two points driving the relationship). However, testosterone concentrations in adult females were also significantly negatively correlated with atrazine. These data suggest a pattern across sexes that are unlikely to be driven by low sample size. In the following two years atrazine concentrations were lower and no effect on testosterone occurred. This suggests that increased atrazine concentration is related to decreased testosterone, opposite to what the authors argue. We believe that Solomon et al. (2008), as review writers, should critically evaluate studies regardless of whether they report or fail to report effects.

69	744	"The conclusion of Murphy et al. (2006b) was that there were no consistent effects on plasma hormone concentrations along an atrazine exposure concentration gradient."	X	Positive	Murphy et al. 2006b	A likely reason why there were not consistent effects in the Murphy et al. (2006b) is because they compare adults across years during the late summer in 2002 and early summer in 2003. Sex hormone concentrations are known to differ seasonally due to changes in reproductive physiology. Therefore, we expect to find different hormonal patterns during different times of the year. It is not appropriate to attempt to find consistency across years if animals are not collected at comparable times or reproductive cycles. All of the authors on the Solomon et al. (2008) paper except for James A. Carr are also authors on the Murphy et al. (2006b) manuscript and thus they were aware of this problem.
70	744	"There were no changes in the concentration of vitellogenin in plasma, which is an estradiol-dependent response (Spanó et al. 2004). This result indicates that the changes observed in plasma concentrations of the steroid hormones were either transitory, in error, or not sufficiently great to cause adverse effects on reproductive function of <i>C. auratus</i> ".	X	Positive	Spanó et al. 2004	These fish were only exposed for 21 days and we have no information describing the way in which estradiol increased over those 21 days or for how long it had been elevated before the end of the study. Therefore, we do not know if estradiol was elevated for an appropriate amount of time to illicit a response in plasma vitellogenin. A more sensitive measure would have been to evaluate vitellogenin concentration or gene expression in the liver where it is synthesized. Indeed, we also have no information on whether this increase in estradiol is transient, so concluding that it was transient is unfounded. Finally, the insinuation that a single endpoint known to be sensitive to estradiol is enough evidence to conclude that there were no functional changes due to increased estradiol is odd.
71	744	"Laregemouth bass (<i>Micropterus salmoides</i>), approximately 2 y of age, were exposed to technical atrazine at nominal concentrations of 0, 25, 35, 50, 75, or 100 µg/L in the water column for 20d during the nonreproductive season."	X	Positive	Gross et al. 1997 meeting abstract/ presentation	This citation is a meeting abstract, yet Solomon et al. (2008) dedicate almost a page to describing the results of this study and even include a figure. We should not treat scientific meeting presentations as peer reviewed articles.
72	744	Results for female bass indicated an inconsistent, non-concentration-dependent response for testosterone in plasma with no statistically significant differences between control and treatments.	X	Positive	Gross et al. 1997 meeting abstract/ presentation	This is one of many examples of Solomon et al.'s promotion of the idea that there are not consistent patterns in the atrazine data both within and among studies. In this case, there was a consistent pattern. Atrazine consistently had no effects on testosterone concentration in female Largemouth bass.
73	744	"No statistically significant responses in plasma vitellogenin concentrations, which are a sensitive estradiol-dependent response in fish, were observed, which suggests that there were no functional changes due to the small, inconsistent, and transient changes in plasma estradiol concentrations."	X	Positive	Gross et al. 1997 meeting abstract/ presentation	See comment (#70) above for Spanó et al. (2004). Here fish were exposed for only 20 days.

74	744-745	"...but there was no concentration-dependent relationship and the statistically differences were not consistent so no overall conclusions could be made."	X	Positive	Gross et al. 1997 meeting abstract/ presentation	This comment directly contradicts the quote made by Solomon et al. (2008) "The 11-ketotestosterone response in male fish appeared to be a threshold response." In addition, this is one of many examples of Solomon et al.'s (2008) repeated attempts to dismiss significant treatment effects because there is not a linear and monotonic dose response.
75	745	"...2 or 20 µg [atrazine]/L resulted in changes in response to the priming effect of ovulated female salmon urine, changes in androgen secretion, and changes in steroid concentrations in the bile (Moore and Waring, 1998)...Increased metabolic activity is a common response to exposure to environmental pollutants as well as being a natural phenomenon in the recrudescence cycle. Thus, it cannot be excluded as a possible cause for the observed response."	X	Positive	Moore and Waring 1998	If there is a treatment effect in a controlled experiment we must assume that the cause of a significant effect was the treatment, in this case atrazine. There is a 5% chance that atrazine did not induce the endpoints measured in this study ($\alpha=0.05$). The physiological mechanisms through which these effects occurred (i.e. increased metabolic activity) is simply fodder for discussion and future research and in no way influences the result that atrazine caused several reproductive changes in this study.
76	745	"Based on the available information, the only evidence for effects of atrazine on concentrations of steroid hormones in blood plasma are those reported by Hayes et al. (2002)."	X	Positive	Hayes et al. 2002	This quote is exactly the same sentence as on page 739 (see #55 and #66)
77	745	"...Carr et al. (2003) randomly selected animals that represented the entire range of body sizes from each tank. In contrast, Hayes et al. (2002) systematically selected animals that completed metamorphosis early. Whether differences in body size confounded their analysis is unknown."	X	Positive	Carr et al. 2003 and Hayes et al. 2002	Upon reading Hayes et al. (2002), we can find no evidence to support the claim that they systematically selected animals that completed metamorphosis early. However, neither of these studies corrected for body size and thus laryngeal size in both studies are confounded with body size. High variation in body size would make recording a true significant difference in laryngeal size extremely difficult. Body size correction is simple and only involves the use of an ANCOVA instead of an ANOVA.
78	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (Allran and Karasov, 2001; Hayes et al. 2002, 2003; Sullivan and Spence, 2003; Jooste et al. 2005; Carr et al. 2003; Orton et al. 2006; Kloas et al. 2008)."	X	Positive	-	Only four out of eight of these studies provide data on metamorphosis. Orton et al. (2006) claim no significant effects but also provide a $p<0.05$. Three out of four studies show a significant decrease in size at metamorphosis with atrazine exposure and the other, fifteen out of 16 studies (excluding cases with contaminated controls) show significant reductions in amphibian size with atrazine exposure (Rohr and McCoy 2010).

79	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (Allran and Karasov, 2001; ...)."	X	Positive	Allran and Karasov 2001	Allran and Karasov (2001) did not quantify the effects of atrazine on thyroid function. Further, effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.
80	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Hayes et al. 2002, 2003;...)."	X	Positive	Hayes et al. 2002	Hayes et al. (2002) did not quantify the effects of atrazine on thyroid function. Effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.
81	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Hayes et al. 2002, 2003;...)."	X	Positive	Hayes et al. 2003	Hayes et al. (2003) did not present any data on the effects of atrazine on metamorphosis.
82	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Sullivan and Spence, 2003;...)."	X	Positive	Sullivan and Spence 2003	Sullivan and Spence (2003) did not quantify the effects of atrazine on thyroid function. Effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.
83	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Jooste et al. 2005;...)."	X	Positive	Jooste et al. 2005	Jooste et al. (2005) did not present any data on the effects of atrazine on metamorphosis.
84	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Carr et al. 2003;...)."	X	Positive	Carr et al. 2003	Carr et al. (2003) did not quantify the effects of atrazine on thyroid function. Effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.
85	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Orton et al. 2006;...)."	X	Positive	Orton et al. 2006	Orton et al. (2006) did not quantify the effects of atrazine on thyroid function. Effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.
86	746	"In general, studies examining the effects of atrazine on metamorphosis suggest no consistent concentration related effects of atrazine on thyroid function (...Kloas et al. 2008)."	X	Positive	Kloas et al. 2008	Kloas et al. 2008 did not quantify the effects of atrazine on thyroid function. Effects on time to metamorphosis or size at metamorphosis do not accurately indicate proper or improper thyroid function.

87	746	"Several studies have shown no effects of atrazine on the time to metamorphosis (...Kloas et al. 2008)."	X	Positive	Kloas et al. 2008	In Kloas et al. (2008), no statistics were conducted for atrazine effects on time to metamorphosis, no measures of within-group variance were provided for time to metamorphosis, and the graphs for time to metamorphosis are indiscernible.
88	746	"Several studies have shown no effects of atrazine on the time to metamorphosis (Hayes et al. 2002, 2003...)."	X	Positive	Hayes et al. 2003	Hayes et al. (2003) did not present any data on the effects of atrazine on metamorphosis.
89	746	"Several studies have shown no effects of atrazine on the time to metamorphosis...in <i>R. pipiens</i> (Allran and Karasov, 2000...)."	X	Positive	Allran and Karasov 2000	While it is true that Allran and Karasov (2000) conclude that there were no effects on time to metamorphosis, we need to be cautious drawing this conclusion because they only used 50% of their metamorphs in this analysis without describing how they selected this subset of metamorphs or why they only used 50% for this analysis but used 100% of the metamorphs for the size at metamorphosis analysis.
90	746	"Several studies have shown no effects of atrazine on the time to metamorphosis...in <i>R. pipiens</i> (...Orton et al. 2006)."	X	Positive	Orton et al. 2006	Orton et al. (2006) claim that there was no significant effect of atrazine on developmental rate but they also provide a $p < 0.05$. They offer no within-group variance estimate for the developmental rate data and thus there is no way to determine whether the effect is significant. Thus, we cannot conclude that there was no effect.
91	746	"These effects are difficult to interpret considering that individual concentrations of atrazine both accelerated (20 µg atrazine/L) and slowed (320 µg atrazine/L) time to metamorphosis to a small degree, whereas concentrations between 40 µg atrazine/L and 160 µg atrazine/L had no effect on metamorphosis (Sullivan and Spence 2003)."	X	Positive	Sullivan and Spence 2003	A regression analysis revealed a significant positive relationship between atrazine concentration and time to metamorphosis. A multiple comparison analysis revealed that no adjacent concentrations were significantly different from one another and thus there is no evidence of non-monotonicity. Thus, the pattern is not difficult to interpret. Even if it was non-monotonic, it would not necessarily make it difficult to interpret.
92	747	"Overall, there are no consistent reported effects of atrazine on metamorphosis in fish or amphibians."	X	Positive	-	There are very consistent effects on size at or near metamorphosis (see case #78 above)

93	747	"these results suggest that atrazine does not consistently affect thyroid function in developing amphibians."	X	Positive	-	Only two studies have directly examined the effects of atrazine on thyroid hormones in amphibians (Larson 1998 and Freeman et al. 2005) and both showed significant effects of atrazine on thyroid function for all three amphibian species tested. So, the effects of atrazine on thyroid function thus far are consistent, although only a small number of amphibian species have been tested. Once again, quantifying metamorphic traits, such as timing to metamorphosis or size at metamorphosis, does not provide accurate and direct information on thyroid function.
94	747	"They conclude that atrazine does not directly affect corticosterone at the concentrations tested."	X	Positive	Larson et al. 1998	While this statement is somewhat true, it is not completely accurate. Larson et al. (1998) state, "The model also indicates an indirect form of endocrine disruption (as defined by Crisp et al. 1998), in that the contaminant [atrazine] does not appear to be mimicking either corticosterone or thyroxine but instead is modifying an environmental cue that triggers the production of the hormones." Hence, Larson et al. (1998) are suggesting that atrazine is behaving like a "stressor", rather than a hormone mimic, that stimulates alterations in these hormones and consequently metamorphic traits. Larson et al. (1998) conclude, and their data support, that atrazine is causing changes in corticosterone and thyroxine hormones in <i>Ambystoma maculatum</i> that affect their metamorphic traits.
95	747	"Exposure of carp (<i>Cyprinus carpio</i>) to 100 µg atrazine/L for 72h or less resulted in greater plasma cortisol concentrations (Gluth and Hanke, 1985). This effect was dependent upon exposure temperature,..."	X	Positive	Gluth and Hanke 1985	Gluth and Hanke (1985), as cited in the literature cited of Solomon et al. (2008), did not test effects of temperature on responses to atrazine.
96	747	"Interestingly, similar cortisol responses were observed after 72h of exposure to a wide variety of contaminants..."	X	Positive	Gluth and Hanke 1985	The fact that atrazine induces a stress response that has also been demonstrated across a wide variety of contaminants does not negate the fact that it is a stressor that could be problematic for the animals or (potentially) their populations. In fact, Solomon et al. (2008) are suggesting that atrazine is indeed a "contaminant" and thus causes negative affects.
97	747	" <i>Salmo salar</i> exposed to 84ug atrazine/L also exhibited hyperglycemia and anorexia (Nieves-Puigdoller et al.2007), suggestive of a stress response."	X	Positive	Nieves-Puigdoller et al. 2007	Again, Solomon et al. (2008) are dismissing significant effects of atrazine as being a "stress response". The fact that atrazine induces a stress response is important and should not be dismissed. The additional effects of hyperglycemia and anorexia are indicative of severe health effects that could lead to death and possibly have population level consequences.

98	748	"gonadal development was not assessed in this study."	X	Positive	Hayes et al. 2006a	Gonadal development was assessed. However, sexual differentiation was not sufficiently progressed to evaluate pesticide effects on gonadal development.
99	748	"The experimental design in this study was seriously flawed. It is possible to test interactions between two or three substances using the isobologram approach but more complex mixtures required ray or multifactorial design (McConkey et al. 2000)."	X	Positive	Hayes et al. 2006a	This statement implies that Hayes et al. (2006a) is attempting to test for interactions among pesticides using an "isobologram approach", which is a popular method for evaluating drug interactions. However, Hayes et al. (2006a) does not attempt to evaluate interactions as evidenced by the following quote from their abstract "Although it cannot be determined whether all the pesticides in the mixture contribute to these adverse effects or whether some pesticides are effectors, some are enhancers, and some are neutral, the present study revealed that estimating ecological risk and the impact of pesticides on amphibians using studies that examine only single pesticides at high concentrations may lead to gross underestimations of the role of pesticides in amphibian declines."
100	748	"That the nine-component mixture caused a greater response is because the mixture was nine times more concentrated than individual components."	X	Positive	Hayes et al. 2006a	This statement can not be substantiated (see Hayes et al. 2006a, quote above #99). Indeed, the nine pesticides were mixed at exceedingly low concentrations (0.1 µg/L each) leading to a final concentration that many, if not most, scientists and the lay public would assume to be safe.
101	748-749	"Because of problems in the experimental design, it is not possible to determine whether the observed effects were the result of potency addition, response addition, synergism, antagonism, or a combination of all of these (LeBlanc and Wang, 2006)."	X	Positive	Hayes et al. 2006a	The Hayes et al. (2006a) experimental design was not intended to test for these differences and the authors do not attempt to determine these differences.
102	749	"Concentrations of atrazine have been reported to exceed 20 µg/L only on rare occasions, even directly after application (Battaglin et al. 2000; Solomon et al. 1996; Giddings et al. 2005)."	X	Positive	-	Solomon et al. (2008) provide numerous citations where atrazine has exceeded 20 µg/L. The USGS National Water Quality Assessment database also has many cases in streams where atrazine exceeded 20 µg/L. Many amphibians inhabit pond environments which tend to have higher atrazine concentrations than streams and are under sampled relative to the extensive USGS efforts in streams. The expected environmental concentration based on the EPA GEENEC v2 software is consistently around 100 µg/L (depending on parameters), indicating that this concentration would be "expected" in ponds near agriculture. All these data suggests that 20 µg/L might not be rare.

103	749	"These concentrations [20 µg/L] typically occur only for a short time in environments that are contiguous to agricultural lands"	X	Positive	-	Solomon et al. (2008) report atrazine half-lives that are regularly over 100 days. These reported half-lives are highly inconsistent with the idea that atrazine concentrations only occur for a short time period. We need to be somewhat cautious because much of the data are from streams where there is regular water flow and water replenishment.
104	749	"Large concentrations of atrazine have been reported to alter adrenal steroid secretion in vitro in frogs (>1000 µg/L) and fishes (84µg/L), but responses are inconsistent between species and may be a general stress response to large exposures that would be rarely found in the environment."	X	Positive	Various	It is unclear why Solomon et al. (2008) choose to conclude the section on stress physiology with this sentence, implying that only <i>in vitro</i> studies have been conducted, that only high concentrations have been studied, and that studies are not consistent. Indeed, several studies have found that atrazine increases cortisol or corticosterone concentrations in vivo in fish and amphibians (e.g. Gluth and Hanke, 1985; Nieves-Puigdoller et al. 2007; and Hayes et al. 2006a). In addition, the implication that 84 µg/L of atrazine is extremely high is inaccurate.
105	750	"A significant limitation with this data set is that it was normalized and reported as percent of control where 100% is arbitrarily assigned to both of the water control groups (i.e., infected and uninfected). Therefore, it is not possible to determine the change in spleen cellularity due to parasitic infection."	X	Positive	Christin et al. 2003	Christin et al. (2003) provide separate data on spleen cellularity pre- and post-infection and thus you can clearly assess the change in spleen cellularity due to parasite infection (author Fig. 1). Solomon et al. (2008) misunderstood this study. There were not infected and uninfected frogs, as they claim. All the frogs received the same amount a nematode larvae. The only factor that differed was the pesticide exposure.
106	750	"Table 2: Head kidney macrophages chemiluminescence response"	X	Neutral	Cossarini-dunier et al. 1988	Cossarini-dunier et al. 1988 did not quantify this response variable. This result was reported in Cossarini-dunier et al. 1987 Journal of Fish Biology, 31 (supplement A), 67-73.
107	751	"A significant confounding factor to this set of experiments is that both Con A or PHA produced very weak induction of T cell proliferation...The modest proliferation responses suggest that both ConA and PHA have weak mitogenic activity in frog T cells or that the conditions for this assay have not been optimized...due to the extremely poor level of stimulation with each of the mitogens, it is difficult to evaluate the biological relevance of the decreased proliferation in the presence of the pesticide exposure."	X	Positive	Christin et al. 2003	The level of stimulation is meaningless to the test. The same amount of ConA and PHA were used alone and in combination with atrazine, so whether the stimulation was weak or strong in inconsequential. In fact, Solomon et al. (2008) contradict themselves by criticizing Brodtkin et al. (2007), who coupled an immune stimulant (thioglycollate) with atrazine, for not testing concentrations of atrazine without the immune stimulant. "Although 8 d of atrazine exposure markedly suppressed leukocyte recruitment into the peritoneal cavity of thioglycollate-stimulated frogs, there was no assessment of the response of frogs to a range of concentrations of atrazine alone. (page 753)." In the former case, Solomon et al. (2008) claim the immune stimulant didn't work well enough, whereas in the latter case they suggest they shouldn't have used the immune stimulant at all. As long as the study is controlled and randomized, the effectiveness of the immune stimulant should not be of concern unless an unnatural immune response was being stimulated.

108	751	"Table 3: <i>Xenopus laevis</i> Viability of splenocytes Decrease Christin et al. 2004"	X	Negative	Christin et al. 2004	The mixture containing atrazine did not decrease splenocyte viability. There was no effect on splenocyte viability.
109	752	"no statistically significant pesticide-associated treatment effects were observed on phagocytosis by spleen-derived phagocytic cells"	X	Positive	Christin et al. 2003	Exposure to the mixture containing atrazine (210 µg/L) significantly reduced the number of phagocytic splenocytes relative to controls post-infection, making this statement inaccurate. Christin et al. (2003) state "21 d after an infection challenge, infected animals previously exposed to a high concentration of pesticides remained unable to respond adequately to mitogens."
110	752	"no statistically significant pesticide-associated treatment effects were observed on the prevalence of lung infection by <i>R. ranae</i> ."	X	Positive	Christin et al. 2003	This is a true but not a completely accurate statement. The mixture containing atrazine did not significantly affect prevalence of <i>R. ranae</i> but it did significantly increase the abundance of <i>R. ranae</i> . However, the results for abundance were published in a follow-up study by Gendron et al. (2003), which Solomon et al. (2008) did not mention. In addition, regression analysis likely would have picked up a significant relationship between atrazine concentration in the mixture and <i>R. ranae</i> prevalence.
111	752	"There was also a decrease in phagocytic activity in spleen cells from <i>R. pipiens</i> , which occurred in the absence of a decrease in spleen cellularity. This is contradictory to the results from an identical study in 2003 (Christin et al. 2003) where no effect on phagocytic activity was observed at any concentrations of the pesticide mixture."	X	Positive	Christin et al. 2003, 2004	Christin et al. (2003) did report significant effects on phagocytic activity. The pesticide mixture, at 210 µg/L of atrazine, reduced phagocytic activity in post-infection animals (author Fig. 3). Therefore, the Christin et al. 2003 and Christin et al. 2004 studies are not contradictory.
112	752	"In 60-d exposure, treatments did not produce scorable plaques (reasons not reported)."	X	Positive	Houck and Session 2006	The reasons that the plaques were not scorable are discussed in the Discussion section of the paper.
113	752	"The methods used in this study were poorly reported, small numbers of animals were used, and the same assay endpoints were not reported for the two exposure periods. Thus the results are essentially uninterpretable..."	X	Positive	Houck and Session 2006	The methods were poorly reported. However, the small number of animals used does not make the study uninterpretable. The sample size was large enough to detect significant effects. Hence, the sample size was sufficient. Second, the fact that the same endpoints were not used in the two separate experiments does not make the results uninterpretable. Researchers regularly quantify different endpoints in different experiments.

114	752	"The only immunological parameter investigated was the number of eosinophils in circulating blood. In light of the fact that the author's goal was to draw a linkage between chemical exposure and decreased immune competence, it is puzzling that information concerning immune status of the frogs was not included in the paper."	X	Positive	Kiesecker 2002	What is puzzling is how in the first sentence of this quote Solomon et al. (2008) state that Kiesecker (2002) did quantify and provide information on the immune parameter eosinophil abundance in frogs, but in the second sentence they claim that Kiesecker (2002) did not provide any information on frog immune status. The first sentence is accurate and the second is inaccurate. Kiesecker (2002) did quantify and provide information on eosinophil abundance.
115	752	"Blood samples for immunological analyses were taken after infestation of frogs with cercariae, making it impossible to determine whether the observed effect on the immune response was due to the infection or herbicide exposure."	X	Positive	Kiesecker 2002	This is indeed a possibility, but it cannot explain the elevated trematode infections with atrazine exposure and thus this hypothesis is not consistent with all the results of Kiesecker (2002). In science, we predominantly proceed with a hypothetico-deductive method. That is, we eliminate hypotheses until one is left standing. Kiesecker (2002) conducted a controlled experiment where the only thing that differed among treatments was pesticide exposure and he revealed an increase in trematode loads and a decrease in immunity associated with exposure to atrazine. Trematodes causing a decrease in immunity cannot explain the increase in trematodes in the atrazine-treated animals relative to the controls; furthermore, amphibians up-regulate immunity in response to an infection. Hence, the most parsimonious explanation is the one that Kiesecker (2002) provided, atrazine suppressed immunity which increased the trematode infections.
116	752	"This effect was "discovered" because of unexpected infections in frogs with <i>F. menigosepticum</i> and was not related to a specific hypothesis test, nor was the study designed to test this hypothesis."	X	Positive	Hayes et al. 2006a	This statement suggests that there is something inherently wrong with discovering something in an experiment not intended to find that particular discovery. Some of the greatest discoveries in science were revealed serendipitously!
117	753	"There is no indication of whether this was a response to infections, the cause of infection, or whether the incidence of thymic plaques increased at greater concentrations, nor was the experiment designed to elucidate this."	X	Positive	Hayes et al. 2006a	Hayes et al. (2006a) conducted a controlled experiment where the only thing that differed among treatments was pesticide exposure and they revealed an increase in <i>F. menigosepticum</i> infections and an increase in thymic plaques associated with exposure to the pesticide mixture. <i>F. menigosepticum</i> causing an increase in thymic plaques cannot explain the increase in <i>F. menigosepticum</i> in the pesticide-treated animals relative to the controls; furthermore, amphibians often up-regulate immunity in response to an infection. Hence, the most parsimonious explanation is the one that Hayes et al. (2006a) provided, pesticides modulated immunity which increased the <i>F. menigosepticum</i> infections.

118	753	"The frogs employed in this study were caught in the wild and there was no knowledge of prior exposure to environmental contaminants"	X	Positive	Brodkin et al. 2007	These frogs were used in a controlled manipulative experiment where they were randomly assigned to treatments and thus it is unclear why previous exposure is relevant. If anything, field caught frogs provide more ecological relevance than inbred, laboratory-reared <i>Xenopus laevis</i> , a species that is profoundly different from most frog species. In fact, if the frogs in this experiment had previous exposure to atrazine and there were any adverse effects of atrazine, it would only select for animals that were more resistant to atrazine exposure, biasing the results toward not observing effects.
119	753	"Because the frogs were not fully immersed, there may have been unquantified differences in exposures between replicates as well as between treatments."	X	Positive	Brodkin et al. 2007	The frogs were assigned randomly to treatments and thus any bias in exposure among treatments will be due to chance. Further, an increase in variation in exposure both within and among treatments will only add random variation to the experimental results that will reduce statistical power and make it more difficult to detect effects of treatments. Hence, this exposure design would reduce, rather than increase, the probability of detecting significant effects of atrazine.
120	753	"This approach is now not commonly employed, especially for assessment of toxicity."	X	Positive	Brodkin et al. 2007	Solomon et al. (2008) provide no citation for the claim that thioglycollate has problems and is now not commonly used. We found no evidence of this anywhere and spoke with immunologist who did not support this claim. A Web of Science search revealed no significant decline in the number of studies using thioglycollate since 1991, and in every year between 2004 and 2008 there were at least 30 studies employing thioglycollate. Thioglycollate will alter both the number and function of immune cells. Thus, Solomon et al. (2008) are correct that it might not be clear whether atrazine affects the number or function of immune cells, but it does not justify dismissing the study. There was a clear immunomodulatory effect of atrazine, regardless of whether it was on cell number, function, or both.
121	753	"The concern is that the thioglycollate activation may confound the assessment of functional responses by macrophages and other phagocytic cells."	X	Positive	Brodkin et al. 2007	There is no confounder in this study. Solomon et al. (2008) are correct that the experiment does not allow us to discriminate the specific cell types that are responding to atrazine, but it is not confounded.

122	753	"Although 8 d of atrazine exposure markedly suppressed leukocyte recruitment into the peritoneal cavity of thioglycollate-stimulated frogs, there was no assessment of the response of frogs to a range of concentrations of atrazine alone, a logical question to ask...these omissions are major flaws of the study."	X	Positive	Brodkin et al. 2007	Solomon et al. (2008) contradict themselves by suggesting that Brodkin et al. (2007) should have eliminated the immune stimulating agent because on page 751 they criticize Christin et al. (2003) for using an immune stimulating agent that did not stimulate the immune response enough (See case # 107 above). Solomon et al. (2008) state "A significant confounding factor to this set of experiments [Christin et al. 2003] is that both Con A or PHA produced very weak induction of T cell proliferation..." So, Solomon et al. (2008) claim the immune stimulant didn't work well enough for Christin et al. (2003), but criticized Brodkin et al. (2007) for using the immune stimulant at all. As long as the study is controlled and randomized, the effectiveness of the immune stimulant should not be of concern unless an unnatural immune response is being stimulated. The lack of a dose response is certainly not a major flaw.
123	753	"In addition, no attempt was made to phenotype the composition of the thioglycollate elicited cell exudates...these omissions are major flaws of the study."	X	Positive	Brodkin et al. 2007	While it would have been nice if Brodkin et al. (2007) phenotyped the cell exudate, this is not a major flaw of the study. It does not confound any effects or alter interpretation in any way.
124	753	"It is unclear whether the effects are directly mediated by atrazine, which cell types are being affected, and/or whether the effects are unique to thioglycollate elicitation."	X	Positive	Brodkin et al. 2007	The effects are due to atrazine ($\alpha=0.05$) because atrazine concentration is the only thing that differed among the treatments. Solomon et al. (2008) are correct that it is not clear which cell types are being affected, but it is clear that atrazine reduced either the number or function of phagocytic cells upon thioglycollate stimulation.
125	753	"[Thioglycollate] is not a common approach to assess the immunotoxicity of an agent on innate immune response"	X	Positive	Brodkin et al. 2007	See case #120 above.
126	754	"The authors reported that baseline levels of atrazine in the tap water reached levels as great as 0.235 μg atrazine/L. Thus, there were no negative control groups employed in the study."	X	Positive	Saglio and Trijasse 1998	Solomon et al. (2008) have been arguing throughout the review that atrazine does not have effects below 20 $\mu\text{g}/\text{L}$. However, here they criticize Saglio and Trijasse (1998) for having concentrations of atrazine in the tap water that Solomon et al. (2008) argue should have no effects on fish. Why are Solomon et al. (2008) criticizing the authors for having low levels of atrazine in the controls unless low levels of atrazine have the potential to induce effects? Is this an indirect admission that 0.235 $\mu\text{g}/\text{L}$ of atrazine could have effects on fish? In addition, several articles authored by Solomon and colleagues have contaminated controls, but those studies are not criticized in this review (e.g. Coody et al. 2004, 2005)

127	754	"Standard deviation measurements were large for the measured endpoints reported to be significantly different from controls, bringing into question whether appropriate statistical tests were used to analyze the data."	X	Positive	Saglio and Trijasse 1998	Solomon et al. (2008) are correct that for some of the response variables it appears that the assumption of homoscedasticity was violated. However, in the cases where the violation of statistical assumptions appears to have occurred, it was because no burst swimming was ever observed in the controls (and thus there was no variation) while burst swimming was common in the atrazine-treated animals. A randomization test would have been more appropriate, but the biological effect appears to be clear.
128	754	"Another issue is the fact that some behaviors in "control" animals differed nearly 15-fold between experiments: a difference that was greater than differences between atrazine treated and control animals in individual experiments. For example, sheltering behavior in "control" animals ranged from 4.63 ± 3.46 to 1.50 ± 1.91 to 15.50 ± 10.28 in three different experiments. Collectively, these issues make the Saglio and Trijasse (1998) paper impossible to interpret."	X	Positive	Saglio and Trijasse 1998	There are two problems with this statement. First of all, it is not valid to statistically compare effects across experiments unless meta-analytical approaches are employed. This is precisely why each experiment has internal controls. The second error is much more serious, however. They provide the correct mean and standard deviations for sheltering behavior, but they failed to mention that in the experiment where the mean was 15.50, chemical cues signaling predation were added to the tanks. Thus, it is not surprising that this mean is much higher than the other two. So, the only comparable means and standard deviations are the 4.63 ± 3.46 and 1.50 ± 1.91 , which would have overlapping confidence intervals and thus would not be statistically different from one another if they were means in a single experiment. Hence, there was consistency in behavioral responses across experiments. There was not 15-fold variation and the paper is not impossible to interpret.
129	754	"The authors reported statistical comparisons of treatment groups relative to the ethanol-treated control when the more appropriate comparisons are relative to fish treated with PGF2a (which was common to all treatment groups). The previously reported effect of atrazine on the priming pheromone response of plasma testosterone, 11-ketotestosterone, and 17,20 β -dihydroxy-4-pregnen-3-one concentrations (Moore and Waring 1998) were not observed with either atrazine or simazine alone or in combination"	X	Positive	Moore and Lower 2001	Solomon et al. (2008) are correct that Moore and Lower (2001) made inappropriate statistical comparisons given their question. However, when the appropriate comparisons are made, the biology seems to indicate (as well as the error bars) that both atrazine and simazine caused significant reductions in milt at all concentrations tested, except at perhaps 0.1 $\mu\text{g/L}$ (author Fig. 2).
130	755	"Inconsistency of responses and incorrect comparisons of treatments make these results difficult to interpret."	X	Positive	Moore and Lower 2001	We found no evidence of inconsistent results within Moore and Lower (2001) or between Moore and Lower (2001) and Moore and Waring (1998). We agree with Solomon et al. (2008) that the statistical comparisons in both Moore and Waring (1998) and Moore and Lower (2001) should have been between the treatments and urine and the treatments and PGF, respectively.

131	755	"Exposure for 30 min to 100 but not 1 or 10 µg atrazine/L reduced the preference/avoidance response ratio to L-histidine"	X	Positive	Tierney et al. 2007	The effect was found at 10 not 100 µg atrazine/L. Thus, the effect was detected at a lower concentration than Solomon et al. (2008) report.
132	756	"The population and reproductive significance of the reported behavioral effects are not clear."	X	Positive	Tierney et al. 2007	If the fish cannot find mates, one would certainly expect there to be reproductive effects. However, we suppose that other cues might also affect mate detection and selection.
133	756	"Rohr et al. (2003) reported that exposure to the greatest concentration (400 µg/L) adversely affected antipredator behavior, which consisted of seeking refuge in response to a potential threat."	X	Neutral	Rohr et al. 2003	Rohr et al. (2003) did not show this. It was Rohr et al. (2004) and there was no obvious threat in this study other than the human taking data.
134	756	"The control of motor patterns in urodeles...is, arguably, relatively primitive compared to mammals"	X	Positive	-	There is no evidence of this, nor is it even relevant to the findings or interpretation of the data.
135	756	"The possible mechanisms underlying these reported effects and whether a direct effect of atrazine on nervous system activity is even plausible in these species are unknown."	X	Positive	Rohr and Palmer 2005	The mechanisms for how atrazine impacts mammals is well understood and even discussed by Solomon et al. (2008). It is certainly plausible that the same mechanism observed in mammals could occur in salamanders, although this is indeed unknown. The mechanisms through which atrazine induces these effects in salamanders should be studied; however our lack of understanding of these mechanisms does not negate the significant effects.
136	756	"Because of our relative lack of understanding of role of behavior in amphibian field biology it is difficult to interpret the potential effects of atrazine, if any, on amphibian behavior."	X	Positive	Rohr and Palmer 2005	First, to claim that we have a lack of understanding of the role of behavior in amphibian field biology is untrue. The role of amphibian behavior in survival and water conservation under field conditions is well understood. Second, it is not difficult to interpret the effects of atrazine on behavior. Rohr and Palmer (2005) demonstrated the long-term effects of atrazine on locomotor activity, social behaviors, and water conserving postures, and also demonstrated a significant increase in water loss with previous atrazine exposure. It might be difficult to interpret the effects of these behaviors and water loss on amphibian populations, but it certainly is not difficult to interpret the effects of atrazine on amphibian behavior.
137	756	"Overall, although several studies have reported effects of atrazine on the olfactory system in salmon, in many cases the responses are inconsistent between studies and do not show clear concentration-related responses."	X	Positive	Saglio and Trijasse 1998, Moore and Waring 1998, Moore and Lower 2001	We found no evidence of inconsistencies among these studies. All three show that atrazine exposure significantly reduced olfactory abilities based on EOG. Furthermore, all three show clear dose responses for the EOGs.

138	756	"Specifically, if the response does not affect the survival, growth, or reproduction of the individuals in a population, there would be no adverse effects on populations."	X	Positive	-	This is not true. For example, if a chemical alters interactions among species, such as between a predator and prey, it can indirectly contribute to population declines or increases, even in the absence of direct effects on survival, growth, or reproduction.
139	757	"the relevance of this [the results of the study] to larger systems and the field should be tempered by the lack of realism in the size of the microcosms and the lack of power inherent in small systems with few replicates"	X	Positive	Rohr and Crumrine 2005	Solomon et al. (2008) are correct that the relevance of this study to conditions in nature should be tempered due to the small size of the microcosms, but this study is probably more ecologically relevant than most of those in Solomon et al.'s (2008) paper given that this study had multiple coexisting species, natural food sources for each species, and was conducted in the field. The lack of statistical power is irrelevant if you detect significant effects. Power is only a concern for non-significant effects.
140	762	"Amphibians inhabiting ponds on agricultural land in Minnesota and exposed to atrazine (0.1-0.5 µg/L) and de-ethyl atrazine (0.1-0.3 µg/L) concentrations 5-fold greater than those reported to produce gonadal effect (Hayes et al. 2003) exhibited no differences in species richness or reproductive success (Knutson et al. 2004). This suggests that, if any gonadal anomalies exist, they do not appear to have effects at the population-level."	X	Positive	-	This does not mean that there are not effects on species richness or reproductive success because these ponds might be sink populations in a metapopulation/metacommunity perspective. That is, their population and diversity might be maintained by immigration from other ponds. Hence, we cannot conclude that there is no population-level effect and that any gonadal anomalies do not impact the population.
141	762	"DuPreez et al. (2005b) examined populations of <i>X laevis</i> inhabiting maize-growing areas with atrazine application versus non-maize-growing areas in South Africa and found no differences in several aspects of population structure including age and size classes."	X	Positive	DuPreez et al. 2005b	Again, this does not mean there are not population-level effects. There could be source populations that maintain the structure in sink populations. There could be evolved resistance to atrazine which could have alternative consequences.
142	763	"Strength of association is best assessed by concentration response."	X	Neutral	-	We live in a world of endocrine-disruption and multiple processes functioning simultaneously within and among organisms, all of which can regularly produce non-monotonic dose responses. Just because monotonic dose-responses were traditionally emphasized in toxicology does not make it right. A concentration response is not necessarily the best way to assess strength of association. Consistency across studies is far more powerful. Plausibility is powerful, but assumes we understand enough about the biology to know what is plausible. Many times this is not the case.

143	764	"The implausible theory that atrazine is bioconcentrated to a large extent in frogs is similarly invoked to explain differences between studies in different laboratories (Hayes 2004)."	X	Positive	Hayes 2004	One does not have to elicit bioconcentration to explain that atrazine is being absorbed by frogs. The bioconcentration potential for atrazine in frogs is around 6, which is indeed low compared to some other chemicals. However, if frogs are continuously detoxifying atrazine at a similar rate as they are taking it in, this could remove atrazine from the water column as a function of frog density. This could especially be the case in studies testing low atrazine concentrations because at high frog densities atrazine could quickly become "limiting". This explanation does not require any implausible theory of excessive bioconcentration on the part of the frogs.
144	764	"There is inconsistency in the effects reported and inconsistency between studies in different laboratories."	X	Positive	-	Various comments above support substantial consistency among studies and laboratories.

^a Any statement that functions to cast doubt on studies that found adverse effects of atrazine were scored as having a positive effect for Syngenta, the company that makes atrazine and funded the Solomon et al. (2008) review. Any case where Solomon et al. describe a significant adverse effect of atrazine on a specific response variable but the primary authors actually reported no effect on this variable was scored as having a negative effect for Syngenta. Any statement which would be expected to have neither a negative or positive effect on Syngenta was scored as neutral.

Table S2. List of studies discussed by Solomon et al. (2008), whether Solomon et al. (2008) describe adverse effects of atrazine at ecologically relevant concentrations from these studies, and whether Solomon et al. (2008) criticize, or cast doubts on the validity of, these studies.

Count	Reference	Page in Solomon et al. (2008)	Effect	Solomon et al. (2008) describe adverse effects of atrazine at ecologically relevant concentrations ^a	Solomon et al. (2008) criticize, or cast doubts on the validity of, the study
1	Morgan et al. 1996	728	Embryotoxicity and teratogenicity	No	No
2	Howe et al. 1998	728	NOEC	No	Equivocal
3	Allran and Karasov 2000	728	Various effects	No	No
4	Allran and Karasov 2001	728	Various effects	No	No
5	Lenkowski et al. 2008	728	Disruption of organ development	No	No
6	Rohr et al. 2004	728	Survival, size at metamorphosis, and behavior	Yes	Yes
7	Rohr et al. 2006	729	Survival	Yes	Yes
8	Napier et al. 1998	729	Malformations in FETAX assay	Yes	Yes
9	Freeman and Rayburn 2004	730	Nuclei abundance	No	No
10	Freeman and Rayburn 2005	730	Developmental rate	Yes	Yes
11	Storrs and Kiesecker 2004	730	Survival	Yes	Yes
12	Fridgen et al. 2005	730	Developmental rate and ovary size	Yes	Yes
13	Hayes et al. 2006a	730	Size at metamorphosis	Yes	Yes

14	Hayes et al. 2002	730	Larval growth, developmental rate, mortality, time to metamorphosis, size at metamorphosis	No	No
15	Carr et al. 2003	730	Larval growth, developmental rate, mortality, time to metamorphosis, size at metamorphosis	No	No
16	Coady et al. 2004	730	Larval growth, developmental rate, mortality, time to metamorphosis, size at metamorphosis	No	No
17	Coady et al. 2005	730	Larval growth, developmental rate, mortality, time to metamorphosis, size at metamorphosis	No	No
18	Kloas et al. in press	730	Larval growth, developmental rate, mortality, time to metamorphosis, size at metamorphosis	No	No
19	Guillette et al. 1994, 1996; Crain et al. 1997, 1999	730	Toxicity and reproductive development	No	No
20	Kiesecker 2002	731-732	Immunity, limb deformities, and larval trematode loads	Yes	Yes
21	Koprivnikar et al. 2006	732	Trematode infectivity in tadpoles	No	Possibly
22	Koprivnikar et al. 2007	732	Trematode infectivity in tadpoles	Yes	Yes
23	Bridges et al. 2004	732-733	Various effects	No	No
24	Beldomenico et al. 2007	734	Decreased weight	Yes	Yes

25	Crain et al. 1997	734	Various effects	No	No
26	Crain et al. 1999	734	Various effects	No	No
27	Coady et al. 2004	734	Female biased sex ratio	No	No
28	Coady et al. 2005	734	Female biased sex ratio	No	No
29	Kloas et al. 2008	734	Female biased sex ratio	No	No
30	Du Preez et al. 2005b	734	Female biased sex ratio	No	No
31	Smith personal comm.	734	Female biased sex ratio	No	No
32	Jooste et al. 2005	734	Female biased sex ratio	No	No
33	Oka et al. 2008	734	Female biased sex ratio	Yes	Yes
34	Suzawa and Ingraham 2008	734	Female biased sex ratio	Yes	Yes
35	Spanó et al. 2004	735	Altered spermatogenesis	No	No
36	U.S. EPA 2005	735	Altered spermatogenesis	Yes	Yes
37	Smith et al. 2005	735	Altered spermatogenesis	No	No
38	Smith 2007 personal comm.	735	Altered spermatogenesis	No	No
39	McDaniel et al. 2008	735	Gonadosomatic indices and altered spermatogenesis	No	No
40	Orton et al. 2006	735	Altered spermatogenesis	Yes (not fully)	Yes
41	Tavera-Mendoza 2001	735	Spermatogenesis and testicular morphology	Yes (not fully)	Yes
42	Tavera-Mendoza 2002a	735	Spermatogenesis and testicular morphology	Yes	Yes
43	Hayes et al. 2002	736	Multiple gonads or hermaphroditism	Yes	Yes
44	Carr et al. 2003	736	Segmented and anomalous gonads	Yes	Yes

45	Coady et al. 2005	736	Testicular ovarian follicles (TOFs)	No	No
46	Du Preez et al. 2008b	737	Testicular ovarian follicles (TOFs)	No	No
47	Jooste et al. 2005	737	Testicular ovarian follicles (TOFs)	No	No
48	Kloas et al. 2008	737	Metamorphosis and sexual differentiation	No	No
49	Oka et al. 2008	737	Testicular ovarian follicles (TOFs)	No	No
50	Hayes et al. 2003	737	Testicular ovarian follicles (TOFs)	Yes	Yes
51	Coady et al., 2004	737	Testicular ovarian follicles (TOFs)	No	No
52	Orton et al. 2006	737	Testicular ovarian follicles (TOFs)	No	No
53	McDaniel et al. 2008	738	Testicular ovarian follicles (TOFs)	No	No
54	Reeder et al. 2005	738	Testicular ovarian follicles (TOFs)	No	No
55	Smith 2007 personal communication	738	Testicular ovarian follicles (TOFs)	No	No
56	Smith et al. 2005	738	Testicular ovarian follicles (TOFs)	No	No
57	Du Preez et al. 2008a unpublished manuscript	738	Testicular ovarian follicles (TOFs)	No	No
58	Pieau et al. 1999	739	Testicular ovarian follicles (TOFs)	No	No

59	Tavera-Mendoza 2002b	739	Oogenesis and ovarian morphology	Yes	Yes
60	U.S. EPA 2005	739	Various effects	No	No
61	Hayes et al. 2006b	739	Nonpigmented ovaries	Yes	Yes
62	Kloas et al. 2008	739	Nonpigmented ovaries	Yes	Yes
63	Cooper et al. 2000	740	Luteinizing hormone and prolactin concentrations	Yes	No
64	Stoker et al. 2002	740	On set of puberty	Yes	No
65	Suzawa and Ingraham 2008	740	Aromatase gene expression (Cyp19A1)	Yes	Yes
66	Kazeto et al. 2004	740	Aromatase gene expression (Cyp19A1 and Cyp19A2))	No	No
67	Hecker et al. 2004	741	Aromatase activity	No	No
68	Murphy et al. 2006b	741	Aromatase activity	No	No
69	Oka et al. 2008	741	Aromatase gene expression	No	No
70	Sanderson et al. 2000	741	Aromatase activity	Yes	Yes
71	Sanderson et al. 2001	741	Aromatase activity	Yes	Yes
72	Hecker et al. 2005a	741	Aromatase activity	No	No
73	Fan et al. 2007	741	Aromatase activity	Yes	Yes
74	Heneweer et al. 2004	741	Aromatase activity	No	No
75	Hayes et al. 2002	742	Plasma testosterone concentration	Yes	Yes

76	Coady et al. 2005	742	Plasma estradiol concentration	Yes	Not fully
77	Hecker et al. 2005a	742	Plasma testosterone concentration	Yes	Yes
78	Kang et al. 1995	742	Plasma testosterone concentration	No	No
79	McDaniel et al. 2008	742	Plasma testosterone and 11-ketotestosterone concentration	No	No
80	Spanó et al. 2004	744	Plasma sex hormone concentrations	Yes	Yes
81	Gross et al. 1997	744	Plasma sex hormone concentrations	Yes	Yes
82	Murphy et al. 2006b	744	Plasma sex hormone concentrations	Yes	Yes
83	Grady et al. 1998	745	Plasma sex hormone concentrations	No	No
84	Moore and Waring 1998	745	Altered priming effect of female urine, androgen secretion, and steroid concentrations in bile	Yes	Yes
85	U.S. EPA 2005	745	Plasma sex hormone concentrations	No	No
86	Hayes et al. 2002	745	Laryngeal dilator muscle size	Yes	Yes
87	Carr et al. 2003	745	Laryngeal dilator muscle size	No	No
88	Coady et al. 2005	745	Laryngeal dilator muscle size	No	No
89	Allran and Karasov 2001	746	Concentration-related effect on thyroid function	No	No
90	Hayes et al. 2002	746	Concentration-related effect on thyroid function	No	No

91	Hayes et al. 2003	746	Concentration-related effect on thyroid function	No	No
92	Jooste et al. 2005	746	Concentration-related effect on thyroid function	No	No
93	Carr et al. 2003	746	Concentration-related effect on thyroid function	No	No
94	Orton et al. 2006	746	Concentration-related effect on thyroid function	No	No
95	Kloas et al. 2008	746	Concentration-related effect on thyroid function	No	No
96	Hayes et al. 2002	746	Time to metamorphosis	No	No
97	Hayes et al. 2003	746	Time to metamorphosis	No	No
98	Carr et al. 2003	746	Time to metamorphosis	No	No
99	Kloas et al. 2008	746	Time to metamorphosis	No	No
100	Sullivan and Spence 2003	746	Time to metamorphosis	Yes	Yes
101	Larson et al. 1998	746	Time to metamorphosis	Yes	Yes
102	Waring and Moore 2004	746	ATPase activity, T3, T4	Yes	No
103	Nieves-Puigdoller et al. 2007	746	Various effects including stress response	Yes	Yes
104	Gluth and Hanke 1985	747	Plasma cortisol and glucose concentration	Yes	Yes
105	Waring and Moore 2004	747	Plasma cortisol and glucose concentration	Yes	Yes
106	Nieves-Puigdoller et al. 2007	747	Plasma cortisol, hyperglycemia, and anorexia	Yes	Yes
107	Larson et al. 1998	747	Plasma corticosterone	Yes	Yes

108	Bisson and Hontela 2002	747	Responsiveness of adrenal cortical tissue to ACTH	Yes	Yes
109	Goulet and Hontela 2003	747-748	Adrenal corticosterone	Yes	Yes
110	Hayes et al. 2006a	748	Plasma corticosterone levels, immunity, and other endpoints	Yes	Yes
111	Pastoor et al. 2008	749	Immunity	No	No
112	Zeeman and Brindley 1981	749	Immunity	Unknown (conc. of effects not provided)	No
113	Cossarinidunier and Hattenberger 1988	749	Immunity	No	No
114	Cossarinidunier 1987	749	Immunity	No	No
115	Cossarinidunier 1988	749	Immunity	No	No
116	Biagianti-Risbourg 1990	749-750	Immunity	Yes	Yes
117	Walsh and Ribelin 1975	749-750	Immunity	Yes	Yes
118	Christin et al. 2003	750-752	Immunity	Yes	Yes
119	Christin et al. 2004	752	Immunity	Yes	Yes
120	Houck and Sessions 2006	752	Immunity	Yes	Yes
121	Kiesecker 2002	752	Immunity	Yes	Yes
122	Hayes et al. 2006a	752-753	Immunity	Yes	Yes
123	Brodkin et al. 2007	753	Immunity	Yes	Yes
124	Saglio and Trijasse 1998	754	Olfaction and behavior	Yes	Yes
125	Moore and Waring 1998	754	Plasma steroids	Yes	Yes
126	Moore and Lower 2001	754-755	Olfaction and hormones	Yes	Yes

127	Moore and Waring 1998	755	Olfaction	Yes	Yes
128	Tierney et al. 2007	755-756	Olfaction	Yes	Yes
129	Rohr et al. 2003	756	Shelter use	Yes	No
130	Rohr and Palmer 2005	756	Behaviors	Yes	Yes
131	Rohr and Crumrine 2005	757	Various effects	Yes	Yes
132	Knutson et al. 2004	762	Amphibian species richness and reproductive success	No	No
133	Du Preez et al. 2005b	762	Population age and size class	No	No
134	Smith 2007, personal communication	762	Population and reproductive parameters	No	No

^a Atrazine concentrations below 500 µg/L were presumed to be "ecologically relevant" because when atrazine was registered, 500 µg/L was approximately its expected environmental concentration based on the Environmental Protection Agencies GENEEC v2 software.

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