Save the Frogs EPA Petition

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Syngenta believes the U.S. EPA should reject the Save the Frogs petition. In summary:

- Atrazine and its potential impacts on amphibian sexual development underwent a detailed evaluation in 2003 by the EPA. This EPA review included several studies cited in the petition summary. The EPA concluded in 2007 that “reproductive fitness (sex ratio, intersex condition) were unaffected” and similar conclusions were reached by other regulatory bodies including Australia Pesticides and Veterinary Medicines Authority in 2008.
- None of the information or references cited in the recent petition justifies a change in the regulatory status of atrazine. There were only a few studies referenced on amphibians and reptiles which were completed after EPA’s 2007 review, and none of these provide compelling scientific evidence that would support this petition.
- Subsequent to the 2007 review by EPA, there have been several relevant publications from independent laboratories which were omitted from the petition that indicate no effects of atrazine on amphibians.
- The majority of the mammalian studies cited in the petition were designed to define mode-of-action, metabolism, and toxicological endpoints. They were not designed to, nor should they be used to, predict risk to aquatic species. The studies consistently demonstrate that effects in mammalian test systems only occur at high-dose levels that are not relevant to environmental concentrations.
- All of the epidemiological studies in the petition have been reviewed by EPA and other regulatory bodies around the world and do not provide evidence of any potential for effects on reproductive health or cancer.

Atrazine and its potential impacts on amphibian sexual development underwent a detailed review and evaluation in 2003 by the EPA. At that time, EPA considered all relevant scientific publications and reports, and included several studies cited in the petition summary as scientific evidence.

After completion of the EPA-required studies (Kloas et al. 2008) to definitively address the potential for atrazine to affect gonadal development in amphibians, EPA once again conducted an evaluation (USEPA 2007). Nearly all of the amphibian and reptile studies cited by Dr. Tyrone Hayes in the petition summary were available to the EPA at that time and were specifically not included in the review because they did not meet EPA's stated design and/or quality standards. EPA concluded in 2007 that "reproductive fitness (sex ratio, intersex condition) were unaffected," and "there is currently no available proof for the hypothesis regarding the purported action of atrazine on the induction of aromatase".

In a similar review in the UK of the cited Hayes 2002 paper, the UK rapporteur in an EU regulatory review stated "the paper grossly overstates the potential effects on frogs and changes in populations based on the data given" (UK 2003). A scientific review of atrazine's potential impacts on amphibians, which included Hayes' publications, by the Australian authorities concluded "that current data indicate that it is unlikely that atrazine is impacting adversely on Australian amphibian populations at current levels of exposure" (APVMA 2008).

No new compelling scientific evidence is provided in the petition summaries by Hayes or Dr. Jason Rohr. Of the 19 amphibian and reptile studies listed in the Hayes and Rohr summaries as supporting this petition, most (12 studies) have already been reviewed or available for review by EPA and/or the EPA Scientific Advisory Panel in 2003 and 2007. None of the references cited in the petition, before or after the 2007 EPA formal review, support the position taken by the petition.

Additional comments and technical reviews of publications can be found in Appendix A.

Save the Frogs EPA Petition – Science Review

Appendix A

Subsequent to the formal EPA review in 2007, several additional papers concerning atrazine's potential effects on amphibians have been published, some of which are identified in the Hayes or Rohr summaries. These include: Hayes et al. (2010), Langlois et al. (2009), Rohr and McCoy. (2010) Rohr et al. (2008), Oka et al. (2008), Skelly et al. (2010), LaFiandra (2008), Storrs and Semlitch, (2008) Spolyarich et al. (2010). A new publication by Tillitt et al.(2010) is also cited (incorrectly) by Hayes relative to effects on fish.

Hayes cites Oka et al. 2008 in the petition summary, stating that this paper supports his claim that "atrazine also completely feminizes" amphibians. This is misleading at best and not supported by the statistical analysis conducted by Oka et al. or their stated conclusions in the paper. These authors conclude that "higher female ratios in atrazine exposure groups in the present study were not caused by the estrogenic action of atrazine, since there is no evidence on induction of P450 aromatase gene in gonad, hepatic VTG induction, and the existence of hermaphroditic gonad."

Three amphibian publications are noticeably missing from the summaries by Hayes or Rohr, presumably because they do not support the claims of the petition. LaFiandra et al (2008). exposed North American treefrogs (*Hyla verisicolor*) to 20 and 200 *µ*g/L atrazine and reported no effects on sex ratio or gonadal development. Likewise, Storrs and Semlitsch (2008) exposed three North American species (*Bufo americanus*, *Hyla versicolor*, and *Rana sphenocephala*) to atrazine (1, 3, or 30 *µ*g/L) and reported no effects on metamorphosis or ovarian development. Spolyarich et al., 2010, report no significant effects on tadpole growth, development and sex ratios of atrazine (0.1, 1, 3 and 30 *µ*g/L) in the spotted marsh frog (*Limnodynastes tasmaniensis.)* These authors concluded that atrazine did not present a significant threat to the normal development of *L. tasmaniensis* larvae in surface waters of irrigated agricultural areas.

Skelly et al. (2010) conducted an analysis of the frequency of green frog (*Rana clamitans*) intersex across a range of land covers (undeveloped, agricultural, suburban and urban). Of the 11 ponds in agricultural areas 9 ponds had corn within their drainage. A total of 233 male frogs were examined for intersex (testicular oocytes). There was no evidence of a positive association with agricultural land cover and intersex. In fact the highest frequencies of intersex were positively associated with suburban and urban landscapes. While this study was not designed to identify the cause of intersex, it supports the many other studies which report no effects on amphibians from atrazine.

The Hayes et al. (2010) publication *Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopus laevis)* purports to demonstrate that atrazine alters the sexual development of amphibians is weakened by design limitations, inconsistency with previous Hayes publications and based on a refuted mode of action.

- Previous Hayes publications were included in reviews on two separate occasions by the USEPA and Scientific Advisory Panels (2003 and 2007) and the studies' hypotheses were not supported. Given that these refuted effects of atrazine on sexual development

are the biological basis and foundation of the current publication, the new effects reported in Hayes et al. (2010) are questionable.

- This publication reports on a study that suffers from serious design limitations, such as including only a single dose level and the lack of a positive control necessary for system validation, comparison of effects, and demonstration of biological plausibility.
- Sampling and statistical methods are not clearly reported in many cases, making evaluation of the study difficult.
- Biological findings are not consistent with previous publications by Hayes.
- Publications not in support of the Hayes et al. 2010 hypothesis are not considered or are inaccurately criticized. Many of the studies cited to support their work (including studies conducted by the lead author) have not withstood rigorous scientific review by independent science panels and regulatory agencies and biological plausibility of key reported effects in this study is not demonstrated.

The Langlois et al. (2008) publication *Low Levels of the Herbicide Atrazine Alters Sex Ratios and Reduces Metamorphic Success in Rana pipiens Tadpoles Raised in Outdoor Mesocosms* suffers from design and conduct limitations and the results do not support the conclusions of the paper.

- The reported effects on sex ratio are likely due to variation in starting sex ratios which deviated widely from the nominal 50:50 male to female ratio and the result of small sample sizes. A relatively small subset of the total available frogs was selected for sex identification. Frogs failing to undergo metamorphosis were also excluded. The reported effects on sex ratio by atrazine exposure are inconsistent with numerous recent publications.
- Reported effects of atrazine and EE2 on mortality and metamorphosis are inconsistent with previous work, including in the case of EE2, with studies conducted in the same laboratory.
- The methods used in this study were not validated by the positive control (EE2), which showed no effects at concentrations where it has been widely demonstrated in numerous studies. The authors' statement that atrazine causes feminization at a concentration of (1.8 µg/L) is therefore not supported. The lack of estrogenic activity by atrazine has been well documented (Eldridge and Wetzel, 2008).
- A number of study design and study conduct limitations and uncertainties impair the strength and utility of this study.
    - The positive EE2 control failed to confirm the sensitivity of the system to feminization via estrogenic compounds, resulting in questionable findings relative to sex ratios.
    - The use of formulated atrazine, rather than atrazine active ingredient, confound the interpretation of the results.
    - The study was conducted with only two concentrations, not enough to properly test the hypotheses stated by the authors.
    - Important physiochemical parameters (eg. pH and dissolved oxygen) were not reported during the first two weeks of the study and therefore cannot be

assessed relative to potential impacts on frogs in this period of the study. When monitored, varying numbers of tank replicates were sampled (between 2 to 5) and treatment means reported, thus tank variability is not provided.

- Given these limitations, the results of this study do not support the conclusion that atrazine alters gonadal differentiation and metamorphosis in developing *R. pipiens.*

<u>The Rohr and McCoy (2010)</u> publication *A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians,* suffers from multiple flaws and limitations and therefore the conclusions are not supported.

- This publication reports on a "qualitative meta-analysis" of existing data. Procedurally, the authors state "We quantify the effects of atrazine on 15 response variables from over 125 studies, and vote counting, the simplest approach to meta-analyses, made it feasible to manage this complexity". Vote counting simply tallies the number of studies in which the authors determined did or did not exhibit an effect of atrazine.
- On the basis of the conclusions of Rohr and McCoy, atrazine is purported to be present at unrealistically high concentrations in the surface waters, and to adversely affect over 12 varying biological endpoints. To have such divergent mechanisms of action all in a single substance and at low exposures would be unprecedented and is without explanation.
- A qualitative analysis is descriptive, with the objective of understanding rather than in drawing conclusions. As such, a qualitative meta-analysis can be used to formulate hypotheses and theories but cannot be used to make conclusions about those hypotheses and theories. The qualitative aspect of the meta-analysis performed by Rohr and McCoy is demonstrated by the fact that there is not an a priory hypothesis of what effects they are considering in their meta-analysis.
- There are several aspects of the paper that are judgmental rather than exploratory or hypothesis generating. For example, the title states "…reveals consistent effects…" which is a conclusion. In fact when they "paraphrase" USEPA's "definition" of meta-analysis as "...a systematic analysis of studies examining similar endpoints to draw general conclusions, develop support for hypotheses, and/or produce an estimate of overall effects", this is really a description of <u>*quantitative*</u> meta-analysis and not <u>qualitative</u> meta-analysis.
- This paper is clearly a biased presentation and one attempting to "…resolve the controversy…" and not explore the evidence which is supposed to be the objective of a qualitative meta-analysis.
- In their qualitative meta-analysis, the authors did not incorporate the effects of differences in species, differences in magnitude of the effects, differences in exposure protocols, differences in experimental environments, differences in endpoints, etc.

In the Rohr et al. (2008) paper, *Agrochemicals increase trematode infections in a declining amphibian species* the authors reported "a significant association between atrazine exposure and elevated frog susceptibility in our mesocosm experiment", however, key data were not measured or provided and their conclusions are inconsistent with expectations from numerous other studies.

- The authors concluded that atrazine, desethylatrazine, and phosphorus were the three best predictors (out of 240 parameters) of larval trematode abundance in northern leapord frogs collected from 18 Minnesota wetlands from March to August of 1999. Based on a 2007 mesocosm study to test a potential causal relationship, the authors reported "a significant association between atrazine exposure and elevated frog susceptibility in our mesocosm experiment." However, key data from the field and mesocosm study were not measured nor provided, thereby questioning the reliability of the authors' causal inferences and overall conclusions that agrochemical exposure is linked to increased trematode infections in amphibians.
- The authors did not provide any supporting raw data from the field or mesocosm studies within the main publication or accompanying Supplementary Information. Independent analysis of these data by interested stakeholders (including the public and regulatory agencies) are critical for evaluating dataset robustness and determining the significance, reproducibility, and reliability of the authors' statistical correlations, path analyses, and/or significance tests.
- Based on an extensive set of laboratory and mesocosm studies for atrazine, direct effects of atrazine on freshwater algal species will not occur at atrazine concentrations (≤0.59 µg/L) measured within the 18 Minnesota wetland sites.
- Contrary to the authors' assertion, desethylatrazine is orders of magnitude less toxic than parent atrazine to freshwater algae.
- Published findings from previous studies by Rohr *et al.*, as well as other researchers, are inconsistent with this study.
- Available data from a comprehensive set of mammalian studies do not support the conclusion that low concentrations of atrazine or desethylatrazine directly affects the immune system of vertebrates, including amphibians.

The Tillitt et al.paper (2010) *Atrazine reduces reproduction in fathead minnow (Pimephales promelas)* reports reductions in spawning and egg production, however study design limitations exist and the reported findings are inconsistent with other known studies.

- The results reported by Tillitt et al. (2010) are inconsistent with the results of 4 fish full life cycle studies which report no effects on reproduction, two of which were for the same species (fathead minnow) of fish.
- These 4 studies were conducted using a standard protocol, accepted by the USEPA. They are of much longer duration (274-450 days) and much higher atrazine exposures (maximum exposures 95 to 2000 ppb) and fish are continuously exposed to the test product from an early life stage (embryo/larval/juvenile) throughout development and reproductive stages until a pre-determined number of spawns occur. Endpoints include:
    - F_0: embryo hatching success, survival, length, weight, # eggs/spawn, total # of eggs, # spawns/female, # eggs/female
    - F_1: hatching success, survival, length, weight

- Overall, although the conduct of this study was sound (in terms of the description of the methods and most of the procedures used), the design of the study and its interpretation are problematic.
- The weaknesses in the design relate to the deviations from the standard protocol in terms of the number of fish per replicate, the subsampling of half the fish at 14 d, and the retention of data from replicates in which one of the fish may have been reproductively compromised at the start of the study.
- These differences in the design do not allow the results of this study to be directly compared to two previous studies which followed the standard protocol and did not report statistically or biologically relevant responses at greater range of exposure-concentrations.
- The effect on production of eggs between 14 and 30 days could have been an artifact of the design and the inclusion of some fish with impaired development of the gonads.
- The authors overstate their results and the mechanism they propose for their alleged effect is not supported by their data. For example, they suggest that atrazine may have affected final oocyte maturation leading to a decrease in the number of eggs yet their own data fail to show an effect on the proportion of
- fish with stage V follicles.
- Considering these points, the weight of evidence suggests that the overall conclusion is that there is either no or only a weak effect of atrazine on production of eggs in fathead minnows.

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