NEIL ABERCROMBIE
Governor

RUSSELL S. KOKUBUN
Chairperson, Board of Agriculture

SCOTT E. ENRIGHT
Deputy to the Chairperson

State of Hawaii
DEPARTMENT OF AGRICULTURE
1428 South King Street
Honolulu, Hawaii 96814-2512
Phone: (808) 973-9600 FAX: (808) 973-9613

TESTIMONY OF RUSSELL S. KOKUBUN
CHAIRPERSON, BOARD OF AGRICULTURE

BEFORE THE HOUSES COMMITTEE
ON
ENERGY AND ENVIRONMENTAL PROTECTION AND HEALTH

MARCH 28, 2013
10:00 A.M.
ROOM 325

HOUSE CONCURRENT RESOLUTION NO. 129
REQUESTING THE DIRECTOR OF HEALTH TO ESTABLISH A TASK FORCE TO STUDY THE EFFECTS OF ATRAZINE ON HUMAN HEALTH

Chairs Lee and Belatti and Members of the Committees:

Thank you for the opportunity to provide testimony on House Concurrent Resolution No. 129 that establishes a task force to study the effects of atrazine on human health. The department appreciates the intent of the resolution but believes it will be duplicating efforts proposed by the U.S. Environmental Protection Agency (EPA) and is therefore unnecessary.

The EPA last concluded its evaluation of atrazine in 2003, evaluating close to 150 published studies investigating a wide array of effects potentially relevant to human health risk assessment. From 2010 – 2011, the EPA presented information to the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) for an independent scientific peer review on over 150 new studies concerning atrazine and its potential impact on human health. SAP is composed of biologists, statisticians, toxicologists and other experts who provide independent scientific advice to the EPA on a wide-range of health and safety issues related to pesticides. At the recommendation of the Scientific Advisory Panel, the Environmental Protection Agency will begin a re-evaluation review of Atrazine in mid-2013.

Therefore while the intent of H.C.R. No. 129 is commendable, the department believes that a more in-depth and thorough review of the best available science research can be provided by the EPA with advice from the SAP. The Department would also defer to the Department of Health.

Thank you for allowing me to present testimony on this resolution.

Island Fresh

NEIL ABERCROMBIE
GOVERNOR OF HAWAII

LORETTA J. FUDDY, A.C.S.W., M.P.H.
DIRECTOR OF HEALTH

STATE OF HAWAII
DEPARTMENT OF HEALTH
P.O. Box 3378
HONOLULU, HAWAII 96801-3378

In reply, please refer to:
File:

COMMITTEE ON HEALTH

COMMITTEE ON ENERGY & ENVIRONMENTAL PROTECTION

H.C. R. 129, REQUESTING THE DIRECTOR OF HEALTH TO ESTABLISH A TASK FORCE TO STUDY THE EFFECTS OF ATRAZINE ON HUMAN HEALTH

Testimony of Loretta J. Fuddy, A.C.S.W., M.P.H.
Director of Health

March 28, 2013
10:00 A.M.

Department's Position: The Department of Health respectfully opposes this measure.

Fiscal Implications: One FTE toxicologist, other programmatic and clerical support which will be approximately $200,000.

Purpose and Justification: HCR 129 requests the Director of Health to establish a task force to study the effects of atrazine on human health. The Department believes a review of the human health effects of atrazine by a local task force is not justified because the Environmental Protection Agency (EPA) is currently undergoing an extensive scientific review of this pesticide. To ensure that the best scientific information is being used, an independent Scientific Advisory Panel (SAP) is advising the EPA on key aspects of the scientific evaluation. The resources of the EPA and the SAP far surpass those of a local task force.

The Department believes that Hawaii-specific monitoring data would assist the state and EPA in evaluating offsite impacts of pesticide use. However, the Department has limited data on air, surface water and near shore impacts of atrazine and other pesticides used in Hawaii. We are currently exploring available resources and partnerships to address this data gap.

Thank you for the opportunity to testify on this measure.

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:36 AM
To: HLTtestimony
Subject: FW: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 9:46 PM
To: EEPtestimony
Cc: karly.williams@gmail.com
Subject: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Karly Williams	Babes Against Biotech	Support	No

Comments:

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

Hawaii Farm Bureau
FEDERATION

2343 Rose Street • Honolulu, Hawaii 96819
Phone: (808) 848-2074 • Neighbor-Islands: (800) 482-1272
Fax: (808) 848-1921 • Email: info@hfbf.org
www.hfbf.org

HOUSE COMMITTEES ON
HEALTH
and
ENERGY AND ENVIRONMENTAL PROTECTION

HCR 129 and HR 100

REQUESTING THE DIRECTOR OF HEALTH TO ESTABLISH A TASK FORCE TO STUDY THE EFFECTS OF ATRAZINE ON HUMAN HEALTH

Thursday, March 28, 2013
10:00 AM
Conference Room 325

Aloha Chairs Belatti and Lee, Vice Chairs Morikawa and Thielen, and Members of the Committees,

I am Dean Okimoto, President of the Hawaii Farm Bureau Federation (HFBF). Organized since 1948, the HFBF is comprised of approximately 2,000 farm family members statewide, and serves as Hawaii's voice of agriculture to protect, advocate, and advance the social, economic, and educational interests of our diverse agricultural community.

HFBF strongly opposes these resolutions because they are unnecessary and because they contain assertions that are grossly inaccurate, misleading, and inflammatory.

Furthermore, to assign our State Department of Health (HDOH) to chair a local task force to study the effects of atrazine on human health is unrealistic and unfair. HDOH simply does not have the resources to conduct a study of this magnitude. Fortunately, a federal agency, the Environmental Protection Agency (EPA), exists to do exactly this type of assessment under federal laws and regulations specific to the use of pesticides. EPA *is* the appropriate agency to study the effects of atrazine on human health, and in fact, the agency has already done this within its rigorous and lengthy pesticide registration process. EPA continues to review new research and information on the compound and is scheduled to conduct a registration review within the next several months.

With regard to the allegations made in these resolutions; it is well known that EPA has reviewed and rejected certain studies alluded to in the resolutions. The studies were rejected because they did not meet EPA's robust criteria for pesticide research. Despite this, certain websites and charismatic speakers continue to cite these flawed studies to rally fear and distrust about the use of atrazine.

Atrazine has been used for many decades in many countries and continues to be used safely. It has been determined to be safe by health organizations and government authorities around the world. While it is true that it is not used in Europe, the assertion is specious since a very similar triazine herbicide *is* used there. Atrazine is an important product in Hawaii, and elsewhere, because it protects crops from competition by weeds that rob them of nutrients and water.

Hawaii's drinking water is regularly tested and it is true that atrazine has been found in some water systems at extremely low levels, many times lower than EPA's very conservative threshold safety levels. These detections are not expected to continue, in part because of strict use controls to prevent leaching and because the use of atrazine in Hawaii has decreased substantially over the last few decades.

Although we strongly oppose these resolutions, if the Committees wish to pursue a local review of this agricultural tool, we respectfully request that the long-standing Pesticide Advisory Committee be assigned the task. This body has the diverse membership and expertise in pesticide matters that will be required.

Thank you for your consideration of our comments and for your continued support of farming in Hawaii.

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:37 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 11:28 PM
To: EEPtestimony
Cc: Scoleman@surfrider.org
Subject: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

HR100
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Stuart Coleman	Surfrider Foundation	Support	No

Comments: My name is Stuart Coleman, and I am the Hawaii Coordinator of the Surfrider Foundation, an environmental non- profit dedicated to preserving the the world's oceans and beaches. I am writing in support of HR 100, which would authorize DOH to form a task force to study the effects of atrazine, one of the most widely used pesticides in the country. This toxic chemical has been banned in the European Union and other countries around the world because it is known to be an endocrine disruptor that causes all kinds of environmental and human health hazards, from chemical castration in frogs to breast and prostrate cancer in humans. Hawaii has shown high levels of atrazine in the water supply; so this resolution would allow DOH to assess how serious a threat this pesticide is to the environment and human health. Mahalo for your consideration. Aloha, Stuart Coleman

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:33 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 4:42 PM
To: EEPtestimony
Cc: gr8bluhron@gmail.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Billie Dawson	Individual	Support	No

Comments: Numerous studies have definitively linked atrazine with endocrine disruption, reproductive damage and cancers in humans and wildlife. Atrazine, a chemical that interferes with natural hormone functions, assaults and affects male sexual development, reproduction and growth of fish, amphibians, wildlife and humans. Atrazine contributes to increased prostate cancer, decreased sperm count and high risk of breast cancer in humans. Extremely persistent in the environment, atrazine is still detectable in France 15 years after its last usage there. The 'smart' European Union has already banned atrazine, due to unacceptable impacts to human health and the environment. Atrazine is a dangerous herbicide; it's use in Hawaii is criminal.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:36 AM
To: HLTtestimony
Subject: FW: *Submitted testimony for HR100 on Mar 28, 2013 10:00AM*

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 8:30 PM
To: EEPtestimony
Cc: watsonblake8@gmail.com
Subject: *Submitted testimony for HR100 on Mar 28, 2013 10:00AM*

HR100
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Blake Watson	Individual	Support	No

Comments:

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:32 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 4:08 PM
To: EEPtestimony
Cc: esfhawaii@hotmail.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Elijah Frank	Ohana O Kaua'i	Support	No

Comments: We need independent study on atrazine. Any study coming from the industry itself cannot be trusted as being unbiased. Atrazine has been proven to be an endocrine disrupter. Atrazine can mimic estrogen at very low doses below FDA MCL's. the biotec h industry also has a habit of donating large funds to any university that decides to do research on pesticides like atrazine. We must watch out for this type of influence on whoever we work with to do our research. Syngenta has spent millions of dollars over the past few years to persuade science to prove low levels of atrazine are safe for humans. Any research by the state must be independent of Syngenta, Monsanto, Dow Chemical, DoPont, BASF, and Bayer. We at Ohana O Kaua'i strongly support independent te sting of atrazine on human health.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Wednesday, March 27, 2013 3:53 PM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM
Attachments: gfk.hayes.3.2013.rohr2010.pdf

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 2:32 PM
To: EEPtestimony
Cc: ofstone@aol.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Jeri Di Pietro	Hawai`i SEED and GMO Free Kaua`i	Support	No

Comments: Aloha Committee members, Please support a study on the health effects of Atrazine in Hawai`i. On Kaua`i citizens have found the presence of atrazine in the well water system at Waimea Canyon Middle School and in homes. It is known that Atrazine is sprayed in the test fields from Polihale to Poipu, and in Puhi, Lihue and Hanamaulu. We have too many cases of miscarriages, cancer and childhood illness. Studies are long overdue. We have 5 of the Big 6 chemical corporations on our island. The chemical field trials have escalated from round up and atrazine to 2,4-d and dicambra, with very little oversight. This tinkering with chemically resistant crops is damaging our image as a world class visitor destination. Please recognize the suffering of our communities that are exposed to this environment on a daily basis. We look to you for assistance in this matter. Mahalo for your joint concern. Jeri Di Pietro

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

A Qualitative Meta-Analysis Reveals Consistent Effects of Atrazine on Freshwater Fish and Amphibians

Jason R. Rohr and Krista A. McCoy

Department of Integrative Biology, University of South Florida, Tampa, Florida, USA

Objective: The biological effects of the herbicide atrazine on freshwater vertebrates are highly controversial. In an effort to resolve the controversy, we conducted a qualitative meta-analysis on the effects of ecologically relevant atrazine concentrations on amphibian and fish survival, behavior, metamorphic traits, infections, and immune, endocrine, and reproductive systems.

Data sources: We used published, peer-reviewed research and applied strict quality criteria for inclusion of studies in the meta-analysis.

Data synthesis: We found little evidence that atrazine consistently caused direct mortality of fish or amphibians, but we found evidence that it can have indirect and sublethal effects. The relationship between atrazine concentration and timing of amphibian metamorphosis was regularly nonmonotonic, indicating that atrazine can both accelerate and delay metamorphosis. Atrazine reduced size at or near metamorphosis in 15 of 17 studies and 14 of 14 species. Atrazine elevated amphibian and fish activity in 12 of 13 studies, reduced antipredator behaviors in 6 of 7 studies, and reduced olfactory abilities for fish but not for amphibians. Atrazine was associated with a reduction in 33 of 43 immune function end points and with an increase in 13 of 16 infection end points. Atrazine altered at least one aspect of gonadal morphology in 7 of 10 studies and consistently affected gonadal function, altering spermatogenesis in 2 of 2 studies and sex hormone concentrations in 6 of 7 studies. Atrazine did not affect vitellogenin in 5 studies and increased aromatase in only 1 of 6 studies. Effects of atrazine on fish and amphibian reproductive success, sex ratios, gene frequencies, populations, and communities remain uncertain.

Conclusions: Although there is much left to learn about the effects of atrazine, we identified several consistent effects of atrazine that must be weighed against any of its benefits and the costs and benefits of alternatives to atrazine use.

Key words: aromatase, behavior, disease, gonads, immunity, metamorphosis, parasite, reproduction, testicular ovarian follicles, vitellogenin. *Environ Health Perspect* 118:20–32 (2010). doi:10.1289/ehp.0901164 available via *http://dx.doi.org/* [Online 23 September 2009]

The herbicide atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is the second most commonly used pesticide in the United States (Kiely et al. 2004) and perhaps the world (Solomon et al. 1996; van Dijk and Guicherit 1999). It is a photosynthesis inhibitor used to control certain annual broadleaf weeds, predominantly in corn but also in sorghum, sugarcane, and other crops and landscaping. The environmental risk posed by atrazine to aquatic systems is presently being reevaluated by the U.S. Environmental Protection Agency (U.S. EPA 2003, 2007). One of the challenges in evaluating the safety of atrazine has been that its biological effects are highly controversial, and much of the debate in the literature has been targeted at its effects on freshwater vertebrates (Hayes 2004; Renner 2004).

There have been four reviews on the biological effects of atrazine, all of which were funded by the corporation that produced or produces this chemical (Giddings et al. 2005; Huber 1993; Solomon et al. 1996, 2008). However, none of the past reviews used a meta-analytical approach to identify generalities in responses to atrazine exposure. Meta-analysis, as paraphrased from the U.S. EPA, is the systematic analysis of studies examining similar end points to draw general conclusions, develop support for hypotheses, and/or produce an estimate of overall effects (U.S. EPA 2009a). This sort of weight-of-evidence approach would provide directional hypotheses for future work on atrazine. Furthermore, it would offer invaluable information to regulatory agencies on general and expected impacts of atrazine on freshwater vertebrates that might help resolve much of the controversy surrounding atrazine. Given the lack of a meta-analytical assessment and the potential importance of any atrazine effects, we set out to conduct an objective, qualitative meta-analysis on the effects of atrazine on amphibian and fish survival, behavior, metamorphic traits, and immune, endocrine, and reproductive systems.

Atrazine Persistence, Transport, and Exposure

To place the results of this meta-analysis within an ecologic context and to evaluate the relevance of studied atrazine concentrations and exposure regimes, we briefly discuss the fate, transport, and field concentrations of atrazine. Atrazine is persistent relative to most current-use pesticides. Ciba-Giegy Corporation (1994), the company that previously produced atrazine, reported no detectable change in atrazine concentration after 30 days in hydrolysis studies conducted at pHs between 5 and 7, and an aqueous photolysis half-life of 335 days under natural light and a neutral pH. Half-lives from field and mesocosm studies are variable because degradation can depend on various environmental conditions. Nevertheless, several field and mesocosm studies report half-lives > 3 months (e.g., de Noyelles et al. 1989; Klaassen and Kadoum 1979).

Atrazine is also relatively mobile—regularly entering water bodies through runoff—and concentrations in surface waters often peak after rains. Several researchers have suggested that atrazine can be transported 1,000 km aerially (van Dijk and Guicherit 1999). Indeed, atrazine has been found regularly in surface waters and precipitation great distances from where it is used, such as above the Arctic Circle, albeit at low concentrations (van Dijk and Guicherit 1999).

Wet deposition of atrazine might also be important in some areas. In a review on atmospheric dispersion of current-use pesticides, van Dijk and Guicherit (1999) reported more studies detecting atrazine in rain or air (from European and U.S. sites) than any other current-use pesticide. The maximum reported wet deposition of atrazine is 154 µg/L from Iowa precipitation (Hatfield et al. 1996). Wet deposition > 1 µg/L was reported regularly in North America and Europe between 1980 and the early 1990s (reviewed by van Dijk and Guicherit 1999). As a reference point, the maximum contaminant level for drinking water set by the U.S. EPA is 3 µg/L atrazine (U.S. EPA 2002).

Surface water is likely the primary source of atrazine exposure for freshwater vertebrates. Data on atrazine concentrations in surface water, however, are more abundant for lotic (streams and rivers) than lentic (lakes, ponds, wetlands, ditches) systems (Solomon et al. 2008), primarily because of the extensive stream

Address correspondence to J.R. Rohr, University of South Florida, Department of Integrative Biology, SCA 110, 4202 East Fowler Ave., Tampa, FL 33620 USA. Telephone: (813) 974-0156. Fax: (813) 974-3263. E-mail: jasonrohr@gmail.com

Supplemental Material is available online (doi:10.1289/ehp.0901164.S1 via http://dx.doi.org/).

We thank the Rohr lab, M. McCoy, and anonymous reviewers for comments on this work.

Funds were provided by grants from the National Science Foundation (DEB 0516227), the U.S. Department of Agriculture (NRI 2006-01370 and 2009-35102-0543), and the U.S. Environmental Protection Agency STAR grant R833835) to J.R.R.

The authors declare they have no competing financial interests.

Received 2 July 2009; accepted 23 September 2009.

monitoring conducted by the U.S. Geological Survey National Water Quality Assessment project and Syngenta Crop Protection, Inc. (U.S. EPA 2007). In lentic systems, water is not replenished as it is in lotic systems, and chemicals can concentrate as lentic systems dry. Maximum reported concentrations in lentic systems are often 2.5–10 times higher than maximum concentrations in lotic systems (Baker and Laflen 1979; Edwards et al. 1997; Evans and Duseja 1973; Frank et al. 1990; Kadoum and Mock 1978; Kolpin et al. 1997). Additionally, many amphibians develop in ephemeral agricultural ponds that might receive and concentrate atrazine (Knutson et al. 2004).

Given the limited data on atrazine concentrations in lentic systems, the expected (or estimated) environmental concentration (EEC) is a reasonable alternative for estimating concentrations to which aquatic organisms are likely to be exposed. GENEEC2 software (U.S. EPA 2009b) calculates standardized EECs used by the U.S. EPA for Tier-1 chemical risk screening. EECs are important because chemical registration decisions entail comparing lowest observable effect concentrations (LOECs) to EECs to determine whether higher-level modeling is warranted. Hence, effects of a chemical near or below the EEC can affect the decision to approve its use.

For present atrazine application rates, EECs based on GENEEC2 software are typically near 100 µg/L but can be higher for some crops. However, the recommended application rates (~ 2 lb active ingredient/acre) are now two to four times less than they were in the early 1990s (~ 8 lb active ingredient/acre). Hence, at the time of atrazine registration, LOECs near or below 500 µg/L, a feasible EEC at the time, might have triggered Tier-2 testing and might have raised concerns about the safety of atrazine that could have compromised its registration. Given both past and present-day conditions, the lack of thorough data on atrazine concentrations in lentic systems, and the common use of agricultural ponds, ditches, and wetlands by amphibians and fish, we suggest that concentrations near or below historical EECs (≤ 500 µg/L) are ecologically relevant when considering the findings of this meta-analysis. This is arguably conservative given that atrazine concentrations > 500 µg/L have been regularly recorded in agricultural ponds and ditches (Baker and Laflen 1979; Edwards et al. 1997; Evans and Duseja 1973; Frank et al. 1990; Kadoum and Mock 1978; Kolpin et al. 1997).

Methods

We selected studies for this meta-analysis beginning with those cited by Solomon et al. (2008), the most recent review of atrazine effects on amphibians and fish. We then supplemented these studies by searching Web of Science (Thomson Reuters, New York, NY) to identify studies that might have been missed by Solomon et al. (2008). The search terms were "atrazine" combined with either "amphibian*" or "fish*".

Selection criteria for inclusion of studies in meta-analyses can affect the conclusions that are drawn (Englund et al. 1999). Hence, we excluded from this meta-analysis studies that had substantial contamination in control treatments or reference sites (unless a regression approach was taken to analyze the data); no presentation of statistics and within-group variance estimates; considerable inconsistencies that could affect the biological conclusions; spatial confounders associated with atrazine treatments; pseudoreplication; or other considerable flaws in experimental design. We evaluated whether the exclusion of these studies changed the conclusion of the meta-analysis for each end point (Englund et al. 1999). For the 15 response variables, the inclusion of studies that did not meet our criteria never altered the conclusions of our meta-analyses, and in some cases including these studies actually strengthened the conclusions. Because of this and space limitations, studies that were excluded and why, as well as the directions of effects in these studies, are provided in Supplemental Material available online (doi:10.1289/ehp.0901164.S1 via http://dx.doi.org/).

To conduct a qualitative meta-analysis, we chose to use the vote-counting method—in which we tallied the number of studies that did and did not detect effects of atrazine—for several reasons. We quantified the effects of atrazine on 15 response variables from > 125 studies, and vote counting, the simplest approach to meta-analyses, made it feasible to manage this complexity. Vote counting also facilitates identifying response variables that might warrant more sophisticated meta-analyses based on effect sizes. Finally, we chose vote counting because it is a conservative approach, biasing results toward detecting no overall effect (Gurevitch and Hedges 1993). Because most atrazine studies conducted analysis of variance to test for dose responses, despite regression analyses providing much greater statistical power (Cottingham et al. 2005), we include studies that had substantial trends for effects of atrazine (i.e., a nonsignificant increase or decrease) with studies that reported statistically significant effects ($\alpha = 0.05$). Our criteria for a trend were a clear dose response, a probability value < 0.1, or authors interpreting their nonsignificant result as a trend. Never did including trends change our conclusions of the meta-analysis.

Results and Discussion

Effects of atrazine on fish and amphibian survival. Many researchers have evaluated the effects of atrazine on fish (reviewed by Giddings et al. 2005; Huber 1993; Solomon et al. 1996) and amphibian survival (e.g., Allran and Karasov 2000, 2001; Brodeur et al. 2009; Diana et al. 2000; Freeman and Rayburn 2005; Rohr et al. 2003, 2004, 2006b). Our general conclusions from these studies are consistent with the conclusions of authors from previous atrazine reviews (Giddings et al. 2005; Huber 1993; Solomon et al. 1996, 2008): There is not consistent, published evidence that ecologically relevant concentrations of atrazine are directly toxic to fish or amphibians. There are, however, some important exceptions (e.g., Alvarez and Fuiman 2005; Rohr et al. 2006b, 2008c; Storrs and Kiesecker 2004). Because our conclusions are consistent with previous reviews, we did not conduct a meta-analysis on survival.

Effects of atrazine on fish and amphibian development and growth. **Background on metamorphosis.** A basic understanding of four concepts about amphibian metamorphosis is necessary to interpret the effects of any chemical on time to, or size at, metamorphosis. First, amphibians must reach a minimum size before they can metamorphose (Wilbur and Collins 1973). Second, once they reach this size, they can accelerate development and metamorphose earlier if they are in a stressful environment or metamorphose later if they are in a good environment (Wilbur and Collins 1973). Last, metamorphosis is predominantly controlled by corticosterone and thyroid hormones (Larson et al. 1998); thus endocrine system disruption can lead to inappropriately timed metamorphosis.

These important facts have profound implications for understanding the effects of pollution on metamorphic traits. For example, imagine that an amphibian shunts energy away from growth to detoxify a chemical and, as a result, reaches the minimum size for metamorphosis 5 days later than amphibians not exposed to the chemical. Once this amphibian reaches the minimum size for metamorphosis, it might accelerate its developmental rate and metamorphose 5 days earlier to get out of the stressful chemical environment. In this example, there is no net effect of the chemical on time to metamorphosis despite inarguably having considerable effects on energy use, growth, and development (Larson et al. 1998). A single chemical could delay, accelerate, or have no effect on timing of metamorphosis, depending on chemical type and concentration.

This example highlights four points. First, a lack of an effect of a chemical on timing of metamorphosis does not mean there was no effect on developmental rate or hormones that drive metamorphosis, as concluded by Solomon et al. (2008). Second, nonmonotonic dose responses in the timing of metamorphosis are expected and are likely common. This is because there are several processes occurring (detoxification, growth, and modulation of developmental timing) that can be temporally

offset and that likely have different (and potentially opposite) functional responses to the same chemical. Third, timing of metamorphosis in response to chemicals should be highly variable. This variation should not be interpreted as inconsistencies across studies (e.g., Solomon et al. 2008), because the complexity of metamorphosis is expected to induce extreme variability. Finally, unlike timing of metamorphosis, size at metamorphosis is expected to monotonically decrease with increasing chemical concentration across species and studies (controlling for time to metamorphosis) because energy used for detoxification is often taken away from that used for growth and development.

Effects on metamorphic traits. Our qualitative meta-analysis on the effects of atrazine on metamorphic traits is consistent with the predictions described above. Twelve of 21 studies found significant effects of atrazine on metamorphic timing, with 7 showing an increase and 7 showing a decrease in time to metamorphosis; thus, as predicted, the direction of the effect was not consistent across studies (Table 1). Seven of the 21 studies had either clear nonmonotonic dose responses or were possibly nonmonotonic (Table 1). These results are consistent with the high variability and high probability of nonmonotonicity expected for this end point.

Only two studies explicitly quantified the effects of atrazine on both thyroid hormones and timing of metamorphosis, and both showed significant nonmonotonic effects (Freeman et al. 2005; Larson et al. 1998) (Table 1). Further, Larson et al. (1998) revealed delays in growth and development early in life followed by accelerated development and early metamorphosis once a critical size for metamorphosis was reached. Additional studies that quantify the impacts of atrazine on thyroid hormones, corticosteroid hormones, and changes in growth and development through time are needed.

In contrast to timing of metamorphosis, size at metamorphosis shows a clear dose-dependent response to atrazine exposure (Table 1). Fifteen of 17 studies and 14 of 14 species showed significant reductions, or considerable trends toward reductions, in amphibian size at metamorphosis associated with atrazine exposure, and all of these studies reported effects at ecologically relevant concentrations based on the above criteria (Table 1). Similar growth reductions have been observed in fish (Alvarez and Fuiman 2005; McCarthy and Fuiman 2008). Atrazine consistently reduced amphibian size, which is likely to have adverse effects on amphibian populations because smaller metamorphs generally have lower terrestrial survival, lower lifetime reproduction, and compromised immune function (Carey et al. 1999; Scott 1994; Smith 1987). However, population-level effects of atrazine have not been empirically tested for in nature and thus need to be evaluated explicitly.

Effects of atrazine on fish and amphibian behavior. **Effects on locomotor activity.** Twelve of 13 studies reported that atrazine exposure increased amphibian or fish locomotor activity over at least a portion of the concentration gradient tested (Table 2). Interestingly, 4 of 5 studies on fish, but none of the studies on amphibians, reported nonmonotonic dose responses. For fish, low concentrations of atrazine stimulated hyperactivity, but higher concentrations caused reductions in activity. For amphibians, hyperactivity was typically observed at the concentrations tested, but higher concentrations would likely eventually become toxic and reduce activity. All studies conducted on fish detected effects of atrazine on locomotor activity, whereas 88% of the studies on amphibians detected atrazine effects (Table 2).

The effects of atrazine on amphibian and fish locomotor activity are consistent with atrazine-induced changes in locomotor activity in mammals. Atrazine seems to cause hyperactivity in mammals by competing with receptors for the inhibitory neurotransmitter gamma-aminobutyric acid, by altering monoamine turnover, and through neurotoxicity of the dopaminergic system (Das et al. 2001; Rodriguez et al. 2005). One study showed that atrazine has similar effects on the nervous system of Ranid frogs (Papaefthimiou et al. 2003), but additional studies are needed that evaluate the mechanisms responsible for atrazine-induced activity changes in fish and amphibians.

Effects on antipredator behaviors. Six of 7 studies reported that atrazine decreased amphibian and fish behaviors associated with predation-related risk reduction (Table 2). Reduced predation avoidance behaviors can increase predation risk, whereas increased hyperactivity should increase encounter rates with predators (Skelly 1994). Hence, reduced risk-reduction behaviors coupled with hyperactivity are expected to increase predation. However, there are no published studies on the effects of atrazine on predator–prey relationships of which we are aware. Given that atrazine might have effects on both predators and prey, the effects of atrazine on predator–prey interactions are difficult to predict without additional studies.

Effects on olfaction. Five of 5 studies reported that atrazine exposure reduced olfactory sensitivity of fish in a dose-dependent manner (Table 2). In contrast, 3 of 3 studies on amphibians detected no effects of atrazine on olfaction at much higher concentrations than were tested on fish (Table 2). One study on amphibians stained activated olfactory neurons with agmatine and found no difference in the stimulation of olfactory neurons between atrazine-treated and control animals (Lanzel 2008).

Effects on other behaviors. One study showed that atrazine reduced amphibian water-conserving behaviors, which increased their rate of water loss (Rohr and Palmer 2005) (Table 2). Interestingly, both the hyperactivity and the reduced water-conserving behaviors occurred hundreds of days after atrazine exposure had ceased; there was no evidence that these end points recovered from atrazine exposure, suggesting permanent effects (Rohr and Palmer 2005). Amphibians are extremely susceptible to desiccation; thus atrazine-induced changes in water conserving behaviors would be expected to increase mortality risk.

Effects of atrazine on fish and amphibian immunity and infections. **Effects on immunity.** Our qualitative meta-analysis revealed that atrazine exposure consistently reduced immune functioning of fish and amphibians, with 16 of 18 studies finding effects at ecologically relevant concentrations. However, many of the end points (16 of 39) were from studies where atrazine was tested as part of a mixture of pesticides, and thus the effects of atrazine were not isolated (Table 3). Nevertheless, atrazine exposure—alone (21 of 27 end points) or in a pesticide mixture (12 of 16 end points)—was associated with reduced immune functioning, resulting in an overall reduction in 77% (33 of 43) of the quantified fish and amphibian immune end points (including trends for a decrease) (Table 3). These results are somewhat conservative because in one study multiple genes associated with immunity were significantly down-regulated (Langerveld et al. 2009), but they were counted as a single end point (Table 3).

Effects on infections. Similar to the effects of atrazine on amphibian and fish immunity, atrazine exposure was consistently associated with an increase in infection end points in fish and amphibians at ecologically relevant concentrations (Table 4). Atrazine elevated trematode, nematode, viral, and bacterial infections (Table 4). Of the studies with sufficient statistical power and without obvious confounders, 12 of 14 of the infection end points increased or showed a strong trend toward increasing, indicating either more infected individuals, more infections per individual, faster maturation, or greater reproduction of the parasite within the host, or greater parasite-induced host mortality (Table 4). As with immunity, these patterns should be considered with caution because many of these end points (6 of 16) came from studies where atrazine was part of a mixture of pesticides tested. Nevertheless, atrazine exposure, alone (4 of 7 end points) or in a pesticide mixture or field study (9 of 9 end points), was associated with an increase in infection end points (Table 4). In general, high concentrations of atrazine seem to be directly toxic to trematodes and viruses, possibly reducing infection risk for amphibians

(Forson and Storfer 2006a; Koprivnikar et al. 2006; Rohr et al. 2008b), whereas more ecologically common concentrations seem to increase amphibian susceptibility, elevating infection risk (Forson and Storfer 2006b; Gendron et al. 2003; Kiesecker 2002; Rohr et al. 2008c).

Several atrazine studies collected immunologic data only from animals that were also exposed to parasites, thus confounding immune parameters with parasite exposure and loads (Christin et al. 2003; Forson and Storfer 2006b; Gendron et al. 2003; Hayes et al. 2006; Kiesecker 2002; Rohr et al. 2008c). However, in each of these studies, atrazine was associated

Table 1. Summary of the results for the effects of atrazine on the developmental rate and size at or near metamorphosis for amphibians.

	Net effect on developmental rate				Size at or near metamorphosis								
Taxon, species	Effect direction	Conc where effect was observed (µg/L)	Nonmonotonic dose response	Excluded from meta-analysis?	Effect direction	Conc where effect was observed (µg/L)	Nonmonotonic dose response	Excluded from meta-analysis?	Conc tested (µg/L)	Atrazine grade	Experiment type	Exposure duration	Reference
Frog													
Bufo americanus	ND	–	NA	No	↓	200	NA	No	200	Comm; Aatrex[a]	PE	≤ 88 days	Boone and James 2003[b]
B. americanus	↓[c]	250, 500, 1,000	Yes	No	↓[d]	No Conc differed from controls	No	No	250, 500, 1,000, 5,000, 10,000	Tech	SR	3 weeks	Freeman et al. 2005
B. americanus	ND	–	No	No	No data	–	No data	Yes	1, 3, 30	Tech	SR	LTM	Storrs and Semlitsch 2008
Rhinella arenarum	↑ at 100 and 1,000, ↓ at 5,000	100, 1,000, 5,000	Yes	No	No data	–	No data	Yes	100, 1,000, 5,000	Tech	SR	LTM	Brodeur et al. 2009
Hyla chrysoscelis	↑	192	No	No	No data	–	No data	Yes	96, 192	Tech	PE, two pulses	≤ 129 days	Briston and Threlkeld 1998[b]
Hyla versicolor	ND[e]	–	Possibly	No	↓	200, 2,000	No	No	20, 200, 2,000	Tech	PE	Mean of 13 days	Diana et al. 2000[f]
H. versicolor	ND	–	NA	No	No data	–	No data	Yes	1, 3, 30	Tech	SR	LTM	Storrs and Semlitsch 2008
Rana clamitans	↓	10	Yes	No	↓	10	Yes	No	10, 25	Tech	SR	≤ 273 days	Coady et al. 2004[f]
Rana pipiens	Unknown[g]	–	No	Yes	↓[h]	Not tested	No	No	20, 200	Tech	SR	LTM	Allran and Karasov 2000
R. pipiens	ND	–	NA	No	↓	0.1	NA	No	0.1	Tech	SR	LTM	Hayes et al. 2006
R. pipiens	ND	–	NA	No	ND	–	NA	No	5	Not provided	SR	ETM, ≤ 45 days	Bridges et al. 2004[i]
Rana sphenocephala	ND	–	NA	No	↓	200	NA	No	200	Comm; Aatrex[a]	PE	≤ 57 days	Boone and James 2003[b]
R. sphenocephala	ND	–	NA	No	No data	–	No data	Yes	1, 3, 30	Tech	SR	LTM	Storrs and Semlitsch 2008
Rana sylvatica	No data	–	No data	Yes	↓	Unknown; conc in ponds not provided	NA	No	3, 30	Comm	FS	Unknown	Kiesecker 2002[j]
Xenopus laevis	No data	–	No data	Yes	ND	–	No	No	1, 10, 25	Tech	SR	Mean of 56 days	Carr et al. 2003
X. laevis	ND	–	NA	No	No data	–	No data	Yes	1, 10, 25	Tech	SR	ETM	Du Preez et al. 2008
X. laevis	↑	100, 450, 800	No	No	Unknown[k]	–	Unknown	Yes	100, 450, 800	Tech	SR	4 weeks	Freeman and Rayburn 2005
X. laevis	Unknown[l,m,n]	–	Unkown	Yes	↓[o]	0.01, 1, 100	Possibly	No	0.01, 0.1, 1.0, 25, and 100	Tech	SR	≤ 75 days	Kloas et al. 2009
X. laevis	↓ detected by regression	No Conc differed from controls	No	No	↓	20, 40, 80, 160, 320	No	No	20, 40, 80, 160, 320	Tech	SR	LTM	Sullivan and Spence 2003
X. laevis	No data	–	NA	Yes	↓	400	NA	No	400	Tech	SR	LTM	Langerveld et al. 2009
Salamander													
Ambystoma barbouri	↑	40, 400	No	No	↓	400	No	No	4, 40, 400	Tech	SR	Mean of 52 days exposure	Rohr et al. 2004
Ambystoma macrodactylum	↑	184	No	No	↓	184	No	No	1.84, 18.4, 184	Tech	SR	30 days	Forson and Storfer 2006a
Ambystoma tigrinum	↑	16 vs. 1.6, but not vs. 0	Possibly; no data	No	ND; trend toward ↓[p]	–	No data	No	1.6, 16, 160	Tech	SR	LTM	Forson and Storfer 2006b
Ambystoma maculatum	↑ and ↓[q]	250	Yes	No	↓	250	No	No	75, 250	Tech	SR	86 days	Larson et al. 1998
A. maculatum	↓	200	NA	No	↓	200	NA	No	200	Comm; Aatrex[a]	PE	≤ 57 days	Boone and James 2003[e]
Ambystoma texanum	↓	200	NA	No	↓	200	NA	No	200	Comm; Aatrex[a]	PE	≤ 88 days	Boone and James 2003[b,r]

Abbreviations: ↓, decreased; ↑, increased; Comm, commercial; Conc, concentration; ETM, embryo to metamorphosis, or earlier (cases where amphibians metamorphosed before atrazine exposure ceased); FS, field survey; LTM, early larvae to metamorphosis; NA, not applicable (used when there were too few concentrations to evaluate nonmonotonicity); ND, not detected; PE, pulse experiment; SR, static renewal experiment; Tech, technical. Excluded studies are listed in Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).

[a]Aatrex is 59.2% inactive ingredients. [b]Community-level study. [c]Authors show that atrazine modifies the thyroid axis for both *X. laevis* and *B. americanus*. [d]All five atrazine concentrations tested reduced frog size relative to controls, but no within-group variance estimates were provided. [e]200 ppb developed faster than 2,000 ppb. [f]Only a single egg mass; might not reflect general response. [g]Use only 50% of the metamorphs in the time to metamorphosis analysis without describing how they selected this subset of metamorphs or why they used only 50% for time to metamorphosis but 100% of the metamorphs for size at metamorphosis. [h]Authors report an interaction between atrazine and time for frog length, indicating that control animals were larger than those exposed to atrazine by the end of the experiment. [i]Tested as a mixture of 5 µ/L atrazine and 5 µ/L carbaryl. [j]Compared ponds with and without atrazine; effects might be due to other factors. [k]Frogs lose weight at metamorphosis, thus mass measurements were confounded by grouping tadpole and metamorph weights. [l]Provide no within-group variance estimate. [m]No statistics provided but conclude that there was no effect of atrazine. [n]Graphs for developmental rate through time are indiscernible. [o]Detected effects in only one of two experiments and for females only. [p]$p = 0.080$ for regression analysis, one-tailed test. [q]Results depended on developmental stage; authors showed that atrazine modifies thyroxine and corticosterone hormones. [r]Results depended on drying conditions.

with both reduced immune parameters and elevated parasite loads. The elevated infections associated with atrazine cannot be explained by parasites reducing immune responses. Hence, the parsimonious explanation for both of these findings is that atrazine reduced immune responses, which elevated infections, especially given that it is often beneficial for vertebrates to up-regulate immunity upon infection (Raffel et al. 2006).

Despite the apparent consistency in the effects of atrazine on immunity and infections

Table 2. Summary of the results for the effects of atrazine on fish and amphibian behaviors.

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Nonmonotonic dose response	Atrazine grade	Experiment type	Exposure duration	Reference
Locomotor activity									
Salamander									
A. barbouri	Locomotor activity after disturbance	↑	400	4, 40, 400	No	Tech	SR	37 days	Rohr et al. 2003
A. barbouri	Locomotor activity after disturbance	↑	400	4, 40, 400	No	Tech	SR	Mean of 52 days; LTM	Rohr et al. 2004
A. barbouri	Locomotor activity after disturbance	↑	40, 400	4, 40, 400	No	Tech	SR	Mean of 47 days; LTM	Rohr and Palmer 2005
A. barbouri	Locomotor activity	↑	400	40, 400, 800	No	Tech	PE	4 days	Rohr et al. (unpublished data)
Frog									
R. sylvatica	Locomotor activity	↑	Two doses of 25 separated by 2 weeks	Two doses of 25 separated by 2 weeks	NA	Tech	PE	1 month	Rohr and Crumrine 2005[a]
B. americanus	Locomotor activity	ND	–	201	NA	Tech	PE	4 days	Rohr et al. 2009
X. laevis	Abnormal swimming	↑	25	1, 10, 25	No	Tech	SR	Mean of 56 days, LTM	Carr et al. 2003
H. chrysoscelis	Burst swimming	↑	Positive dose response	96, 192	No	Tech	PE, two pulses	≤ 129 days, LTM	Briston and Threlkeld 1998
Fish									
Carassius auratus	Burst swimming	↑	0.5, 50	0.5, 5, 50	Possibly	Tech	PE	1 day	Saglio and Tijasse 1998
C. auratus	Burst swimming	↑	0.1, 1, 10	0.1, 1, 10	Possibly	Tech	PE	1 day	Saglio and Tijasse 1998
Oncorhynchus mykiss	Locomotor activity	↑	1, 10	1, 10, 100	Yes	Tech	PE	30 min	Tierney et al. 2007
Lepomis cyanellus	Locomotor activity	↑/↓	400 but not 800	40, 400, 800	Yes, only in presence of natural prey	Tech	PE	4 days	Rohr et al. (unpublished data)
Larval *Sciaenops ocellatus*[b]	Locomotor activity and abnormal swimming	↑	40, 80	40, 80	No	Tech	PE	72 hr	Alvarez and Fuiman 2005
Predation-related risk reduction									
Salamander									
A. barbouri	Refuge use	↓, detected with regression	None	4, 40, 400	No	Tech	SR	37 days	Rohr et al. 2003
A. barbouri	Refuge use	↓	400	4, 40, 400	No	Tech	SR	Mean of 52 days, LTM	Rohr et al. 2004
Frog									
R. sylvatica	Refuge use	↓	Two doses of 25 separated by 2 weeks	Two doses of 25 separated by 2 weeks	NA	Tech	PE, two pulses	1 month	Rohr and Crumrine 2005[a]
C. auratus	Grouping	↓	5, 50	0.5, 5, 50	No	Tech	PE	1 day	Saglio and Tijasse 1998
C. auratus	Sheltering in presence of predator cue	↓	5	0.5, 5, 50	Possibly	Tech	PE	1 day	Saglio and Tijasse 1998
C. auratus	Grouping in presence of predator cue	↓	5	0.5, 5, 50	Possibly	Tech	PE	1 day	Saglio and Tijasse 1998
Larval *S. ocellatus*[b]	Predation rates	ND	40, 80	40, 80	No	Tech	PE	72 hr	Alvarez and Fuiman 2005
Olfaction									
Frog									
B. americanus	Chemical detection of food, parasites, and predator cues	ND	–	201	NA	Tech	PE	4 days	Rohr et al. 2009
Salamander									
Plethodon shermani	Chemical detection of food or sex pheromones	ND	–	300	NA	Tech	SR	28 days	Lanzel 2008
P. shermani	Activated olfactory neurons	ND	–	700	NA	Tech	SR	28 days	Lanzel 2008
Fish									
Salmo salar	Olfactory response (electroolfactogram)	↓	2, 5, 10, 20	0.1, 1, 2, 5, 10, 20	No	Tech	PE	30 min	Moore and Waring 1998
S. salar	Olfactory response (electroolfactogram)	↓	1	0.5, 1	No	Tech	PE	30 min	Moore and Lower 2001
S. salar	Olfactory response (electroolfactogram)	↓	0.5, 1	0.5, 1	No	Tech	PE	30 min	Moore and Lower 2001[c]
O. mykiss	Olfactory response (electroolfactogram)	↓	10, 100	1, 10, 100	No	Tech	PE	30 min	Tierney et al. 2007
O. mykiss	Response ratio to L-histidine	↓	10	1, 10, 100	Possibly	Tech	PE	30 min	Tierney et al. 2007
Other behaviors									
Salamander									
A. barbouri	Water-conserving behaviors	↓	40, 400	4, 40, 400	No	Tech	SR	Mean of 52 days; LTM	Rohr and Palmer 2005[d]

Abbreviations: ↓, decreased; ↑, increased; Conc, concentration; LTM, early larvae to metamorphosis; NA, not applicable (used when there were too few concentrations to evaluate nonmonotonicity); ND, none detected; conc, concentration; tech, technical; PE, pulse experiment; SR, static renewal experiment; Tech, technical. Excluded studies are listed Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).
[a]Community-level study. [b]Larval red drum are often found in freshwater, so they were included in this meta-analysis. [c]Mixture of 0.5:0.5 and 1.0:1.0 atrazine and simazine; thus, total concentration of triazine was 1 and 2 ppb, respectively. [d]Increased salamander water loss and thus desiccation risk.

Table 3. Summary of the results for the effects of atrazine, through water column exposure, on fish and amphibian immunity.

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Nonmonotonic dose response[a]	Atrazine grade	Experiment type[b]	Exposure duration	Reference
Salamander									
A. tigrinum	No. of peripheral leukocytes	↓	16, 160	1.6, 16, 160	No	Tech	SR	Until metamorphosis	Forson and Storfer 2006b
Frog									
R. pipiens	Splenocyte viability	ND	–	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003, 2004[a]
R. pipiens	No. of splenocytes	↓, if using appropriate one-tailed test	210	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003, 2004[a]
R. pipiens	No. of phagocytic splenocytes	↓ postinfection	210	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003[a]
R. pipiens	T cell proliferation	↓ in presence of mitogens	2.1, 21, 210	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003, 2004[a]
R. pipiens	T cell proliferation	↓ in absence of mitogens	2.1, 21, 210	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003, 2004[a]
R. pipiens	Absolute no. of phagocytic cells in spleen	↓	2.1, 21, 210	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2004[a]
R. pipiens	No. of thymic plaques	↑, indicating reduced immune capacity[b]	0.1	0.1	NA	Tech	SR	Until metamorphosis	Hayes et al. 2006
R. pipiens	No. of hemolytic plaques representing antibody secreting B cells	↓	1, 10	1, 10	No	Not provided	SR	4 weeks	Houck and Sessions 2006
R. pipiens	No. of lymphocyte from spleen	ND	–	1, 10	Possibly	Not provided	SR	8 weeks	Houck and Sessions 2006
R. pipiens	No. of white blood cells	↓	0.01 to 10	0.01, 0.1, 1, 10	No	Tech	SR	8 days	Brodkin et al. 2007[c]
R. pipiens	No. of highly phagocytic cells	↓	0.01 to 10	0.01, 0.1, 1, 10	No	Tech	SR	8 days	Brodkin et al. 2007[c]
X. laevis	Splenocyte viability	ND	–	2.1, 21, 210, 2,100	No	Tech	SR	21 days	Christin et al. 2004[a]
X. laevis	Splenocyte cellularity	↓	210, 2100	2.1, 21, 210, 2,100	No	Tech	SR	21 days	Christin et al. 2004[a]
X. laevis	Relative no. of phagocytic cells in spleen	↑	21, 210, 2,100	2.1, 21, 210, 2,100	No	Tech	SR	21 days	Christin et al. 2004[a]
X. laevis	Absolute no. of phagocytic cells in spleen	↓	210, 2,100	2.1, 21, 210, 2,100	No	Tech	SR	21 days	Christin et al. 2004[a]
X. laevis	T cell proliferation	ND	–	2.1, 21, 210, 2,100	No data	Tech	SR	21 days	Christin et al. 2003[a]
X. laevis	Downregulation of several genes involved in skin peptide defense	↓	400	400	NA	Tech	SR	Until metamorphosis	Langerveld et al. 2009
X. laevis	Downregulation of several genes involved in blood cell function	↓	400	400	NA	Tech	SR	Until metamorphosis	Langerveld et al. 2009
R. sylvatica	No. of eosinophil from circulating blood	↓	3, 30	3, 30	No	Tech	SR	4 weeks	Kiesecker 2002
R. pipiens	No. of melano-macrophages from liver	↓	< 1 Do not know maximum concentration	Unknown	No	Comm	FS	Unknown	Rohr et al. 2008c[d]
Rana paulustris	No. of melano-macrophages from liver	↓	117	117	NA	Tech	PE	4 weeks	Rohr et al. 2008c
R. paulustris	No. of eosinophil from liver	ND, trend toward decrease; $p = 0.10$	117	117	NA	Tech	PE	4 weeks	Rohr et al. 2008c
R. clamitans	No. of eosinophil from liver	↓	117	117	NA	Tech	PE	4 weeks	Rohr et al. 2008c
R. clamitans	No. of melano-macrophages from liver	ND	117	117	NA	Tech	PE	4 weeks	Rohr et al. 2008c
Fish									
C. auratus	No. of superoxide radical from macrophages of spleen and kidney	↑ 4 and 8 weeks; indicator of oxidative stress	42	42	NA	Tech	SR	12 weeks	Fatima et al. 2007[a]
C. auratus	Plasma lysozyme activity	↑ at 8 and 12 weeks, argued as a reduction in resistance to infection	42	42	NA	Tech	SR	12 weeks	Fatima et al. 2007[a]
C. auratus	Antibody titers against *Aeromonas hydrophila*	↓	42	42	NA	Tech	SR	12 weeks	Fatima et al. 2007[a]
C. auratus	Antioxidant enzyme in spleen (superoxide dismutase)	↓ at 4, 8, and 12 weeks	42	42	NA	Tech	SR	12 weeks	Fatima et al. 2007[a]
Galaxias maculatus	Leucocrit	↓	3, 50	0.9, 3, 10, 50	Possibly	Tech	SR	10 days	Davies et al. 1994
O. mykiss	Proliferative ability of circulating T lymphocytes (ConA)	↓	> 5,000	1,000–10,000	Possibly	Tech	PE	2 days	Rymuszka et al. 2007
O. mykiss	Proliferative ability of circulating B lymphocytes (LPS)	↓	> 5,000	1,000–10,000	Possibly	Tech	PE	2 days	Rymuszka et al. 2007
O. mykiss	Respiratory burst activity of circulating phagocytes	↓	> 2,500	1,000–10,000	Possibly	Tech	PE	2 days	Rymuszka et al. 2007
Liza ramada and *Liza aurata*	Macrophage quality	↓ (cells degenerated)	25–280	Unknown	Unknown	Unknown	Unknown	Unknown	Biagianti-Risbourg 1990[e]
L. ramada and *L. aurata*	Melanomacrophage centers in liver	↑	25–280	Unknown	Unknown	Unknown	Unknown	Unknown	Biagianti-Risbourg 1990[e]
Salmonidae (species not specified)	White blood cells	↓	100–1,000	Unknown	Unknown	Unknown	Unknown	Unknown	Walsh and Ribelin 1975[e]
Salmonidae (species not specified)	Lymphoid organ quality	↓ (evidence of atrophy)	100–1,000	Unknown	Unknown	Unknown	Unknown	Unknown	Walsh and Ribelin 1975[e]
Salvelinus namaycush, Oncorhynchus kisutch	Spleen weight	↓/ no effect	1,500–13,500	Unknown	Unknown	Unknown	Unknown	Unknown	Zeeman and Brindley 1981
S. namaycush, O. kisutch	No. of lymphocytes	↓/ no effect	1,500–13,500	Unknown	Unknown	Unknown	Unknown	Unknown	Zeeman and Brindley 1981

Abbreviations: ↓, decreased; ↑, increased; Comm, commercial; Conc, concentration; FS, field survey; NA, not applicable (used when there were too few concentrations to evaluate nonmonotonicity); ND, not detected; PE, pulse experiment; SR, static renewal experiment, Tech, technical. Excluded studies are listed in Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).
[a]Atrazine was a component of a mixture of pesticides tested, and thus the experiment did not isolate the effects of atrazine. [b]Atrazine alone and every mixture containing atrazine increased thymic plaques. [c]Immune response stimulated by thioglycollate. [d]No quantified factors correlated with atrazine could parsimoniously explain patterns in infection. [e]As reported by Dunier and Swicki 1993; could not obtain original works.

(Table 3), much remains to be learned about the effects of atrazine and other chemicals on parasite–host interactions (Raffel et al. 2008; Rohr et al. 2006a). For instance, we know little about how atrazine-induced changes affect population or community dynamics or most human diseases.

Effects of atrazine on fish and amphibian gonadal morphology. **General morphologic end points.** Sex differentiation is the process by which gonads develop into either testes or ovaries from an undifferentiated or bipotential gonad (Hayes 1998). This process is distinct from reproductive maturation where the differentiated gonad becomes reproductively functional (e.g., undergoes spermatogenesis in males). Determining if atrazine induces changes in gonadal morphology is an important step in evaluating whether it can influence sexual differentiation.

Atrazine consistently affected male gonadal morphology in fish and amphibians (Table 5). Seven of the 10 studies including results on males and females reported strong trends or statistically significant alterations (6 studies) in at least one aspect of general gonadal morphology associated with atrazine exposure. Alterations included discontinuous and multiple testes, sexually ambiguous gonadal tissue, testicular ovarian follicles (TOFs), altered gonadal somatic index (GSI; ratio of gonad weight to body weight), expanded testicular lobules, and spermatogenic tubule diameter (Table 5).

Effects on ovarian morphology are generally less obvious than those on testicular morphology and are typically dismissed without quantification. None of the three studies on fish or amphibians included in our meta-analysis found significant effects of atrazine on ovarian morphology, suggesting that atrazine induces fewer gonadal abnormalities in females than males. However, additional studies are necessary to fully evaluate the effects of atrazine on female gonadal morphology.

TOFs as a natural phenomenon. Jooste et al. (2005) and Solomon et al. (2008) argued that experiments with high numbers of TOFs in control *Xenopus laevis* support the hypothesis that TOFs are normal in some *X. laevis* populations. Although it was argued long ago that some anurans in some environments transition through a hermaphroditic phase during development (Witschi 1929), the literature we reviewed does not argue that adult amphibians commonly have oocytes within testicular tissue or are naturally hermaphroditic (Eggert 2004; Hayes 1998). Indeed, *X. laevis* sexually differentiates (without a transitional/hermaphroditic stage) during the larval period prior to sexual maturation (Iwasawa and Yamaguchi 1984). Thus, cases of gonadal abnormalities in healthy adult *X. laevis* populations should be rare. Given that simultaneous hermaphroditism has not been previously reported in *X. laevis* despite decades of research on their reproductive biology, an equally or more plausible explanation for high numbers of TOFs in control animals (e.g., Jooste et al. 2005; Orton et al. 2006) is exposure to some type of unmeasured endocrine-disrupting contaminant.

Effects of atrazine on fish and amphibian sex ratios. Given that atrazine exposure has been proposed to feminize gonadal development (Hayes et al. 2002, 2003), it might lead to female-biased sex ratios. Many studies, however, have severe methodologic errors, such as contaminated controls or inadequate data reporting [see Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1)], preventing a conclusive synthesis of the effects of atrazine on sex ratios. None of the sex-ratio studies used the most accepted and powerful approaches for testing for changes in sex ratios (e.g.,

Table 4. Summary of the results for the effects of atrazine, through water column exposure, on fish and amphibian parasite infections.

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Nonmonotonic dose response	Atrazine grade	Experiment type	Exposure duration	Reference
Salamander									
A. macrodactylum	Infectivity of ATV	↓	Not provided	1.84, 18.4, 184	Dose response not provided	Tech	SR	30 days	Forson and Storfer 2006a[a]
A. tigrinum	Percentage infected with ATV	↑ at 16 but not 1.6 or 160	16	1.6, 16, 160	Yes	Tech	SR	Until metamorphosis	Forson and Storfer 2006b[b]
A. tigrinum	Viral load	ND; $p = 0.14$	–	20, 200	No	Tech	SR	2 weeks	Kerby and Storfer 2009
A. tigrinum	Mortality due to ATV	↑	Not provided	20, 200	No	Tech	SR	2 weeks	Kerby and Storfer 2009
Frog									
R. pipiens	*Rhabdias ranae* nematode prevalence	ND; trend toward ↑	–	2.1, 21, 210	No	Tech	SR	21 days	Christin et al. 2003[c]
R. pipiens	No. of adult *R. ranae* nematode	↑, clear dose response	21 + 210 > controls, 210 > water control	2.1, 21, 210	No	Tech	SR	21 days	Gendron et al. 2003[c]
R. pipiens	*Chryseobacterium* (Flavobacterium) *menigosepticum* infections	↑	0.1	0.1	NA	Tech	SR	Until metamorphosis	Hayes et al. 2006[c,d]
R. pipiens	*R. ranae* nematode within host migration	Faster	21, 210	2.1, 21, 210	No	Tech	SR	21 days	Gendron et al. 2003[c]
R. pipiens	*R. ranae* nematode maturation and reproduction	Earlier	21, 210	2.1, 21, 210	No	Tech	SR	21 days	Gendron et al. 2003[c]
R. sylvatica	No. of *Ribieoria* sp. and *Telorchis* sp.	↑	3, 30	3, 30	No	Tech	SR	4 weeks	Kiesecker 2002
R. sylvatica	Limb deformities caused by *Ribieoria* sp.	↑ in ponds with atrazine	Ponds with atrazine	Unknown	NA	Comm	FS	Unknown	Kiesecker 2002
R. clamitans	No. of *Echinostoma trivolvis* cercariae	↑	201	201	NA	Tech	SR	2 weeks	Rohr et al. 2008b[e]
R. pipiens	No. of larval trematodes	↑	< 1 Do not know maximum Conc	Unknown	No	Comm	FS	Unknown	Rohr et al. 2008c[f]
R. clamitans	No. of larval *Plagiorchid* trematodes	↑	117	117	NA	Tech	PE	4 weeks	Rohr et al. 2008c
R. clamitans	No. of *Echinostoma trivolvis* cercariae	↓, but amphibians not exposed to atrazine	20, 200	20, 200	No	Comm; Aatrex[g]	PE	Cercariae exposed for 2 hr	Koprivnikar et al. 2006[h,i,j]
Fish									
C. auratus	Mortality due to *Aeromonas hydrophila* challenge	↑	42	42	NA	Tech	SR	12 weeks	Fatima et al. 2007[c]

Abbreviations: ↓, decreased; ↑, increased; ATV, *Ambystoma tigrinum* virus; Comm, commercial; Conc, concentration; FS, field survey; NA, not applicable (used when there were too few concentrations to evaluate nonmonotonicity); ND, not detected; PE, pulse experiment; SR, static renewal experiment, Tech, technical. Excluded studies are listed in Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).
[a]Effect was observed when combining of 1.84, 18.4, and 184 treatments and comparing with controls; effect might be predominantly due to 184. [b]160 ppb was thought to reduce ATV infectivity explaining nonmonotonicity. [c]Atrazine was a component of a mixture of pesticides tested, and thus the experiment did not isolate the effects of atrazine. [d]Saw this effect only when atrazine was mixed with eight other pesticides. [e]Effect was found pooling pesticides and comparing them with control treatments. [f]No quantified factors correlated with atrazine could parsimoniously explain patterns in infection. [g]Aatrex is 59.2% inactive ingredients. [h]Effects could be due to inactive ingredients. [i]Effects could be due to chemicals other than atrazine that might be in the pond water used to make the stock solutions. [j]All LC_{50}s were calculated incorrectly.

Wilson and Hardy 2002). Only four studies, all on *X. laevis*, were of sufficient quality to be included in our meta-analysis, and only one found that atrazine induced a female-biased sex ratio (see Supplemental Material, Table S2 (doi:10.1289/ehp.0901164.S1)].

Effects of atrazine on fish and amphibian gonadal function. Chemicals that alter gonadal development can affect gonadal function, such as germ cell (e.g., spermatogenesis in males) and steroid hormone production (McCoy et al. 2008; McCoy and Guillette, in press), and thus can lead to altered reproductive success.

Effects on testicular cell types. Spermatogenesis is the process through which mature male gametes (spermatozoa) are produced from precursor cells (spermatogenic cells). The relative ratios of different spermatogenic cell types, rather than abundance of spermatozoa alone, is the most sensitive metric of altered spermatogenesis. Unfortunately, few studies on effects of atrazine on spermatogenesis met our inclusion criteria. Two of two studies demonstrated that atrazine was associated with altered spermatogenesis and that several cell types were affected (Table 6). Thus, atrazine appears capable of altering spermatogenesis, but the contexts and generality of these effects cannot be firmly established. Our analysis once again highlights a need for more rigorous investigations.

Effects on sex hormone concentrations. Sex hormone production is an important function of gonads that can be altered by gonadal abnormalities (McCoy et al. 2008). Indeed, altered hormone concentrations are the defining characteristic, in many cases, of endocrine disruption. Six of seven studies on fish and amphibians document strong trends or significantly (five studies) altered sex hormone concentrations associated with atrazine exposure (Table 6). Although many of these studies were conducted in the field and are therefore correlative, the consistency of these results across studies suggests that atrazine alters sex hormone production and should be considered an endocrine-disrupting chemical. A more thorough understanding of the effects of atrazine on hormone concentrations will require more detailed studies that account for the inherent variability of endocrine system processes.

Table 5. Summary of the effects of atrazine on general gonadal morphology.

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Atrazine grade	Experiment type	Exposure duration	Reference
Testes								
Fish								
Pimephales promelas	Testis size corrected for body size	ND	5, 50	5, 50	Tech	SR	21 days	Bringolf et al. 2004[a]
P. promelas	Spermatogenic tubule diameter	↓	250	25, 250	Tech	FT	21 days	U.S. EPA 2005
Frog								
X. laevis	Discontinuous gonads (abnormal segmentation)	↑	25	1.0, 10, 25	Tech	SR	~78 days during larval period	Carr et al. 2003
X. laevis	Ambiguous gonads (not obviously male or female)	↑	25	1.0, 10, 25	Tech	SR	~78 days during larval period	Carr et al. 2003[b]
X. laevis	Testis size corrected for body size	↑	10	10, 100	Tech	SR	48 days	Hecker et al. 2005a[a]
X. laevis	Sperm/area	ND	–	10, 100	Tech	SR	48 days	Hecker et al. 2005a[a]
X. laevis	Testis size corrected for body size	ND	–	1, 25, 250	Tech	SR	36 days	Hecker et al. 2005a[a]
R. clamitans	Testis size corrected for body size	↓ in juvenile males	ND–3.13	ND–3.13[c]	Comm	FS	Unknown	McDaniel et al. 2008[c]
R. pipiens	TOFs (testicular oocytes)	↑ where atrazine was detected in 2003[c]	ND–3.14	ND–3.13[c]	Comm	FS	Unknown	McDaniel et al. 2008[c,d]
Various spp., mostly *R. clamitans*	Discontinuous testes (abnormal segmentation)	ND	–	ND–2[e]	Comm	FS	Unknown	Murphy et al. 2006a
Various spp., mostly *R. clamitans*	Intersex (having testicular and ovarian tissues)	ND	–	ND–2[e]	Comm	FS	Unknown	Murphy et al. 2006a
Various spp., mostly *R. clamitans*	TOFs (testicular oocytes)	↑ in 1 of 2 years in juveniles, positively correlated with max atrazine Conc in that year	ND–0.73	ND–2[e]	Comm	FS	Unknown	Murphy et al. 2006a
R. clamitans	Testis size corrected for body size	↑ in adult males at agricultural sites in 1 of 2 years	ND–250	ND–2[e]	Comm	FS	Unknown	Murphy et al. 2006b[f]
X. laevis	Hermaphroditism (testicular oocytes, intersex, mixed sex)	ND	–	0.1, 1, 10, 100	Tech	SR	~ 65 days during larval period	Oka et al. 2008
Acris crepitans	Intersex or testicular oocytes	Trend for ↑ $p = 0.07$	Atrazine detections	ND–70	Comm	FS	Unknown	Reeder et al. 1998[g]
Ovaries								
Fish								
P. promelas	Ovary size corrected for body size	Trend for ↓	50	5, 50	Tech	SR	21 days	Bringolf et al. 2004[a]
P. promelas	Proportion of oocytes undergoing atresia	ND	–	25, 250	Tech	FT	21 days	U.S. EPA 2005
Frog								
H. versicolor, *R. sphenocephala*	Ovarian developmental stage	ND	–	1, 3, 30[h]	Tech	SR	Through metamorphosis	Storrs and Semlitsch 2008
B. americanus	Ovarian developmental rate	ND	–	1, 3, 30[h]	Tech	SR	Through metamorphosis	Storrs and Semlitsch 2008

Abbreviations: ↓, decreased; ↑, increased; Comm, commercial; Conc, concentration; FS, field survey; FT, flow-through experiment; ND, not detected; SR, static renewal experiment, Tech, technical. Excluded studies are listed in Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).

[a]No test statistics or degrees of freedom are presented; however, means and variances were presented either in the text or in a figure of the article. [b]*Xenopus* are typically sexually differentiated at the gross morphologic level at metamorphosis; individuals in this study exposed to 25 µg/L were so sexually ambiguous they were initially considered intersex (having both testicular and ovarian issues). [c]Atrazine concentration for the nonagricultural reference site during 2003 was reported incorrectly; repeated attempts to contact the authors for clarification have not been forthcoming. [d]When atrazine concentrations were highest (2003), TOFs per individual occurred in higher numbers; TOFs were positively associated with atrazine, nitrate, and quantity of pesticides in a multivariate comparison, suggesting that atrazine is contributing to TOFs. [e]Concentrations were between ND and 2 except on two occasions at one site, when levels were 65 and 250 µg/L. [f]Authors argued that differences in GSI between agricultural and nonagricultural sites cannot be due to atrazine because GSI does not correlate with atrazine concentration; however, they presented no statistics to support this claim. [g]The relationship between detection of atrazine and the presence of one or more intersex cricket frogs approached significance ($p = 0.07$). [h]The actual concentration of the 30-µg/L treatment was 125 µg/L.

Effects on reproductive success. Reproductive success is strongly linked to population persistence and is likely one of the most important end points in toxicologic studies. Five studies that evaluated the effects of atrazine on measures of reproductive success met our meta-analysis requirements (Table 6). Two studies on adult fish, *Pimephales promelas*, found no significant effect of atrazine on number of eggs produced, fertilization success, proportion of hatchlings, or larval development. However, one of these studies (Bringolf et al. 2004) found several nonsignificant, adverse trends (Table 6). Two of three studies on amphibians found no effects of atrazine on hatching success, whereas one showed reduced hatching success and delayed hatching (Table 6). Given the mixed results, the effect of atrazine on reproductive success needs to be studied more thoroughly.

Effects of atrazine on fish and amphibian vitellogenin. Vitellogenin is an egg yolk precursor protein produced in the livers of female fish and amphibians. Estrogens induce vitellogenin synthesis in both males and

Table 6. Summary of the effects of atrazine on gonadal function.

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Atrazine grade	Experiment type	Exposure duration	Reference
Testicular cell types								
Frog								
R. clamitans	Proportion of juvenile males with > 50% tubules containing spermatids and spermatozoa	Lower at agricultural site with highest atrazine concentrations	Range of medians, 0.068–0.78	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a]
R. pipiens	Proportion of juvenile males with > 50% tubules containing spermatids and spermatozoa	Higher at agricultural site with highest atrazine concentrations	0.342 (mean of median concentrations)	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a]
Fish								
P. promelas	Proportion of primary spermatogonia	↑	25, 250	25, 250	Test	FT	21 days	U.S. EPA 2005
P. promelas	Proportion of secondary spermatogonia	Reduced	25, 250	25, 250	Test	FT	21 days	U.S. EPA 2005
Sex hormone concentrations								
Frog								
X. laevis	Testosterone in adult males	↓	25	25	Tech	SR	46 days	Hayes et al. 2002[b]
X. laevis	Testosterone in adult males	ND	–	10, 100	Tech	SR	48 days	Hecker et al. 2005a
X. laevis	Estradiol in adult males	ND	–	10, 100	Tech	SR	48 days	Hecker et al. 2005a
X. laevis	Estradiol in adult males	ND	–	1, 25, 250	Tech	SR	36 days	Hecker et al. 2005b
X. laevis	Testosterone in adult males	↓	250	1, 25, 250	Tech	SR	36 days	Hecker et al. 2005b
X. laevis	Testosterone in females	↓ at agricultural sites, negatively correlated with concentration of atrazine and breakdown product	< 0.1–4.14	< 0.1–4.14	Comm	FS	Unknown	Hecker et al. 2004
X. laevis	Testosterone in males	Negatively correlated with diamino-chlorotriazine concentration (a product of atrazine breakdown)	< 0.1–4.14	< 0.1–4.14	Comm	FS	Unknown	Hecker et al. 2004
X. laevis	Estradiol in females	↓ at agricultural sites, negatively correlated with conc of atrazine and breakdown product	< 0.1–4.14	< 0.1–4.14	Comm	FS	Unknown	Hecker et al. 2004
R. pipiens	Testosterone in juvenile males (2003)	↓ at agricultural sites	Range of medians, 0.380–0.780	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a]
R. pipiens	Testosterone in juvenile males (2003)	Negatively correlated with atrazine concentration	ND–3.13	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a,c]
R. pipiens	11-Ketotestosterone in juvenile males (2003)	Negatively correlated with atrazine concentration	ND–3.13	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a,c]
R. pipiens	Testosterone in adult females (2003)	Negatively correlated with atrazine concentration	ND–3.13	ND–3.13[a]	Comm	FS	Unknown	McDaniel et al. 2008[a,c]
R. clamitans	11-Ketotestosterone to testosterone ratio in adult females (late summer Aug–Sep 2002)	↑ at agricultural sites	Agricultural sites ranged from ND to 250	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	11-Ketotestosterone to testosterone ratio in adult males (late summer Aug–Sep 2002)	↑ at agricultural sites	Agricultural sites ranged from ND to 250	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	11-Ketotestosterone to testosterone ratio in adult males (early summer May 2003)	↑ at agricultural sites	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Estradiol to testosterone ratio in adult females (late summer Aug–Sep 2002)	↑ at agricultural sites	Agricultural sites ranged from ND to 250	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Estradiol to testosterone ratio in adult males (Late summer Aug–Sep 2002)	↑ at agricultural sites	Agricultural sites ranged from ND to 250	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Estradiol to testosterone ratio in adult males (early summer May 2003)	↓ at agricultural sites	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Estradiol to testosterone ratio in juvenile males (Jul 2003)	↑ at agricultural sites	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Testosterone in adult males (early summer May 2003)	↑ at agricultural sites	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Testosterone in juvenile females (Jul 2003)	↑ at agricultural sites	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
R. clamitans	Testosterone in juvenile males (Jul 2003)	↑ at agricultural sites[d]	Agricultural sites ranged from ND to 0.73	ND–250	Comm	FS	Unknown	Murphy et al. 2006b[d]
Fish								
P. promelas	Testosterone female	ND	–	25, 250	Tech	FT	21 days	U.S. EPA 2005
P. promelas	Estradiol female	Trend (up to a 44% ↓)	25, 250	25, 250	Tech	FT	21 days	U.S. EPA 2005[e]
P. promelas	Testosterone male	Trend (up to a 31% ↓)	25, 250	25, 250	Tech	FT	21 days	U.S. EPA 2005[e]
P. promelas	11-Ketotestosterone male	Trend (up to a 47% ↓)	25, 250	25, 250	Tech	FT	21 days	U.S. EPA 2005[e]

continued next page

females *in vivo*, and quantification of vitellogenin is now an accepted screening test for estrogenic effects of chemicals (Scholz and Mayer 2008). None of the five studies (four on fish) found significant effects of atrazine on circulating or whole-body concentrations of vitellogenin [see Supplemental Material, Table S2 (doi:10.1289/ehp.0901164.S1)]. Hence, these data do not support the hypothesis that atrazine is strongly estrogenic to fish.

Effects of atrazine on fish and amphibian aromatase. Cytochrome p450 aromatase catalyzes the conversion of androgens to estrogens in gonads and is critical for maintaining a balance between these sex hormone classes. Hayes et al. (2002) hypothesized that decreases in testosterone associated with atrazine exposure in their study could be driven by an atrazine-induced increase in aromatase and a concomitant increase in the conversion of testosterone and other androgens to estrogens. This hypothesis seemed reasonable because atrazine was known to increase aromatase in human cancer cell lines and in alligator gonadal–adrenal mesonephros (Crain et al. 1997; Sanderson et al. 2000). However, since 2002, several studies have explicitly tested whether atrazine increases aromatase in fish and amphibians, and only one of six studies included in our meta-analysis found that atrazine was associated with increased aromatase gene expression [see Supplemental Material, Table S2 (doi:10.1289/ehp.0901164.S1)].

Effects of atrazine on fish and amphibian populations and communities. Although there are too few studies examining the effects of atrazine on freshwater vertebrate populations to warrant meta-analysis, and virtually all community-level studies infer—rather than test for—indirect effects (Rohr and Crumrine 2005), the effects of atrazine on populations and communities warrants a brief discussion. Any chemical that affects physiology, growth, development, reproduction, survival, or species interactions can affect population and community dynamics (Clements and Rohr 2009; Rohr et al. 2006a). However, the effects of contaminants might not result in immediate population declines because the survivors of chemical exposure frequently have less competition for resources, thus providing density-mediated compensation for adverse effects of the chemical (Rohr et al. 2006b). Demonstrating that a factor is the cause of any population decline is, indeed, incredibly difficult (Rohr et al. 2008a). Rohr et al. (2006b) revealed significant and delayed declines in *Ambystoma barbouri* salamander populations at 4, 40, and 400 µg/L atrazine, above and beyond the counteracting effects of density-mediated compensation. Although this study provided greater ecologic realism than many studies on atrazine, caution should be taken extrapolating these effects to populations in nature because this study was conducted in laboratory terraria. There is certainly a need for controlled studies on the effects of pesticides on wildlife populations.

Several studies have examined the effects of atrazine on amphibian and fish communities (Boone and James 2003; de Noyelles et al. 1989; Kettle 1982; Rohr and Crumrine 2005; Rohr et al. 2008c). Many of these studies reported alterations in fish or amphibian growth and abundance that seem to be caused by atrazine-induced changes in photosynthetic organisms (reviewed by Giddings et al. 2005; Solomon et al. 2008). At ecologically relevant concentrations, atrazine is expected to have a bevy of indirect effects by altering the abundance of periphyton, phytoplankton, and macrophytes (Huber 1993; Solomon et al. 1996). However, none of these studies distinguish between direct and indirect effects of atrazine on fish or amphibians.

There are several field studies comparing amphibian populations or species richness between atrazine-exposed and unexposed habitats (Bonin et al. 1997; Du Preez et al. 2005; Knutson et al. 2004). All of these studies are correlational, and none thoroughly considered or ruled out alternative hypotheses for the observed patterns.

Caveats. We would be remiss not to mention some caveats regarding this meta-analysis. First, a problem with many meta-analyses is the "file-drawer" effect. This refers to the fact that researchers tend to place the results of experiments showing no effects in their file drawer, and many journals tend to publish fewer studies showing no effects than those with effects (Gurevitch and Hedges 1993; Osenberg et al. 1999). This might be less of a problem in studies on pesticides because these chemicals are designed to kill biota; thus in many cases, the null hypothesis might be an effect rather than the absence of one. Additionally, a substantial industry contingent works to ensure that both significant and nonsignificant effects of chemicals get published. Indeed, in the review of atrazine by Solomon et al. (2008), there were approximately

Table 6. *continued*

Taxon, species	End point	Effect direction	Conc where effect was observed (µg/L)	Conc tested (µg/L)	Atrazine grade	Experiment type	Exposure duration	Reference
Reproductive success								
Salamander								
A. barbouri	Proportion hatched and timing of hatching	ND	–	4, 40, 400	Tech	SR	37 days	Rohr et al. 2003
A. barbouri	Proportion hatched and timing of hatching	↓ and delayed hatching	400	4, 40, 400	Tech	SR	Mean of 52 days	Rohr et al. 2004
Frog								
R. pipiens	Proportion hatched	ND	–	2,590–20,000	Tech	SR	10 days	Allran and Karasov 2001
R. clamitans	Proportion hatched	ND	–	2,590–20,001	Tech	SR	10 days	Allran and Karasov 2001
B. americanus	Proportion hatched	ND	–	2,590–20,002	Tech	SR	10 days	Allran and Karasov 2001
Fish								
P. promelas	Eggs per spawning of exposed adults	Trend for a ↓	5	5, 50	Tech	SR	21 days	Bringolf et al. 2004[b]
P. promelas	Number of spawnings of exposed adults	Trend for a ↓	50	5, 50	Tech	SR	21 days	Bringolf et al. 2004[b]
P. promelas	Fertilization success of exposed adults	Trend for a ↓	50	5, 50	Tech	SR	21 days	Bringolf et al. 2004[b]
P. promelas	Proportion hatched and larval development of offspring from exposed adults	ND	–	5, 50	Tech	SR	21 days	Bringolf et al. 2004[b]
P. promelas	Egg production of exposed adults	ND	–	25, 250	Tech	FT	21 days	U.S. EPA 2005
P. promelas	Fertilization success of exposed adults	ND	–	25, 250	Tech	FT	21 days	U.S. EPA 2005
P. promelas	Proportion hatched and larval development of offspring from exposed adults	ND	–	25, 250	Tech	FT	21 days	U.S. EPA 2005

Abbreviations: ↓, decreased; ↑, increased; Comm, commercial; Conc, concentration; FS, field survey; FT, flow-through experiment; ND, not detected; SR, static renewal experiment, Tech, technical. Excluded studies are listed in Supplemental Material, Table S1 (doi:10.1289/ehp.0901164.S1).

[a]Atrazine concentration for the nonagricultural reference site during 2003 was reported incorrectly; repeated attempts to contact the authors for clarification have not been forthcoming. [b]No test statistics or degrees of freedom were presented; however, means and variances were presented either in the text or in a figure of the article. [c]Authors reported no significant correlation between atrazine and sex hormones in their abstract when, in fact, these end points were negatively correlated; contrary to the authors' conclusion, the negative correlations across sexes and age groups reported in their study are unlikely to occur because of a low sample size or sampling error. [d]Authors argued that differences in hormone levels between agricultural and nonagricultural sites cannot be due to atrazine because hormone concentrations do not correlate with atrazine concentration; however, they presented no statistics to support this claim. [e]Low samples sizes (7–8 fish) likely precluded detecting these considerable effects.

63 cases where atrazine had significant adverse effects and 70 cases where atrazine had no significant effects (Rohr JR, McCoy KA, unpublished data), suggesting that the file-drawer effect is unlikely to be strongly biasing submission and publication of nonsignificant atrazine results. However, we cannot completely discount the possibility that the file-drawer effect generated a bias toward greater publication of significant effects of atrazine.

Another admonishment is that some of the end points in this meta-analysis were not independent of one another. For example, we tallied multiple end points from a single study despite the possibility that they might not be entirely independent.

Finally, we must consider the findings of this meta-analysis on atrazine relative to alternative strategies for weed control. If the alternative to atrazine is another chemical, then we should ideally compare the effects of atrazine to the replacement chemical. In fact, atrazine might be less detrimental to freshwater vertebrates than a replacement herbicide. If the alternative to atrazine does not entail a chemical replacement, then the effects revealed here might indeed be disconcerting. However, we also cannot ignore the benefit, if any, that atrazine provides. Interestingly, several studies estimate that atrazine increases corn yields by only 1–3% (reviewed by Ackerman 2007). To adequately evaluate any chemical, we should ideally conduct a thorough cost–benefit analysis that considers the focal chemical and alternatives to its use and is based on comprehensive and accurate knowledge [see Ackerman (2007) for a review and critique of atrazine cost–benefit analyses].

Conclusions

As in past reviews, we found little evidence that atrazine consistently causes direct mortality of freshwater vertebrates at ecologically relevant concentrations, but there is evidence that atrazine might have adverse indirect ecologic effects. However, in contrast to a previous review on atrazine (Solomon et al. 2008), we unveiled consistent effects of atrazine at ecologically relevant concentrations for many other response variables in our meta-analysis. The discrepancy between our findings and the conclusions of previous reviews could be partly a function of differences in criteria for including studies in the group used to draw general conclusions about atrazine effects. Past reviews (e.g., Solomon et al. 2008) did not clearly define their inclusion criteria, did not make it clear which studies affected their conclusions (or how they came to their conclusions), and regularly dismissed significant effects of atrazine.

Here we reveal that, for freshwater vertebrates, atrazine consistently reduced growth rates, had variable effects on timing of metamorphosis that were often nonmonotonic, elevated locomotor activity, and reduced antipredator behaviors. Amphibian and fish immunity was reliably reduced by ecologically relevant concentrations of atrazine, and this was regularly accompanied by elevated infections. Atrazine exposure induced diverse morphologic gonadal abnormalities in fish and amphibians and was associated with altered gonadal function, such as modified sex hormone production. This suggests that atrazine should be considered an endocrine-disrupting chemical. Finally, we do not have a thorough appreciation of the reproductive repercussions of atrazine.

Several end points had enough well-conducted studies to warrant more sophisticated meta-analyses based on effect sizes (e.g., growth, timing of metamorphosis, activity, immunity, infections, gonadal abnormalities). Meta-analyses based on effect sizes can provide parameter and standard errors estimates and thus can be useful for probabilistic risk assessment and for predicting atrazine effects.

Although we found consistent effects of atrazine on freshwater vertebrates, the consequences of these effects remain uncertain. We know little about how atrazine-induced changes in vertebrate growth, somatic development, behavior, immunity, gonadal development, or physiology affect reproduction, populations, gene frequencies, or communities. However, it was Sir Austin Bradford Hill who wisely stated in his address to the Royal Society of Medicine in 1965 that

> All scientific work is incomplete [and] . . . liable to be upset or modified by advancing knowledge. That does not confer upon us freedom to ignore the knowledge we already have, or to postpone action that it appears to demand at a given time. (Hill 1965)

Whatever action is taken in the re-evaluation of atrazine by the U.S EPA, we strongly encourage regulators to consider the consistent effects of atrazine on various taxa and to weigh these effects against any benefits atrazine provides and the alternatives to atrazine use.

Correction

Corrections have been made from the original manuscript published online: Criteria for identifying results showing "substantial trends" has been clarified; the number of studies has been corrected in the text; and the "effect direction" for relevant studies has been corrected in Tables 1, 3, and 5.

References

Ackerman F. 2007. The economics of atrazine. Int J Occup Environ Health 13(4):437–445.

Allran JW, Karasov WH. 2000. Effects of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae exposed in the laboratory from posthatch through metamorphosis. Environ Toxicol Chem 19:2850–2855.

Allran JW, Karasov WH. 2001. Effects of atrazine on embryos, larvae, and adults of anuran amphibians. Environ Toxicol Chem 20:769–775.

Alvarez MD, Fuiman LA. 2005. Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae. Aquat Toxicol 74:229–241.

Baker JL, Laflen JM. 1979. Runoff losses of surface-applied herbicides as affected by wheel tracks and incorporation. J Environ Qual 8:602–607.

Biagianti-Risbourg S. 1990. Contribution a l' du foie de juveniles de muges teleosteens, (muglides) contamines experimentalment par l' atrazine (s-triazine, herbicide): Interet en ectoxicologie [PhD dissertation]. Montpellier, France:University of Perpigan.

Bonin J, Des Granges J, Rodrigue J, Oullet M. 1997. Anuran species richness in agricultural landscapes of Quebec: foreseeing long-term results of road call surveys. In: Amphibians in Decline: Canadian Studies of a Global Problem (Green DM, ed). St Louis, MO:Society for the Study of Amphibians and Reptiles, 141–149.

Boone MD, James SM. 2003. Interactions of an insecticide, herbicide, and natural stressors in amphibian community mesocosms. Ecol Appl 13:829–841.

Bridges C, Little E, Gardiner D, Petty J, Huckins J. 2004. Assessing the toxicity and teratogenicity of pond water in north-central Minnesota to amphibians. Environ Sci Pollut Res Int 11:233–239.

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

Briston CA, Threlkeld ST. 1998. Abundance, metamorphosis, developmental, and behavioral abnormalities in *Hyla chrysoscelis* tadpoles following exposure to three agrichemicals and methyl mercury in outdoor mesocosms. Bull Environ Contam Toxicol 61:154–161.

Brodeur JC, Svartz G, Perez-Coll CS, Marino DJG, Herkovits J. 2009. Comparative susceptibility to atrazine of three developmental stages of *Rhinella arenarum* and influence on metamorphosis: non-monotonous acceleration of the time to climax and delayed tail resorption. Aquat Toxicol 91:161–170.

Brodkin MA, Madhoun H, Rameswaran M, Vatnick I. 2007. Atrazine is an immune disruptor in adult northern leopard frogs (*Rana pipiens*). Environ Toxicol Chem 26:80–84.

Carey C, Cohen N, Rollins-Smith L. 1999. Amphibian declines: an immunological perspective. Dev Comp Immunol 23:459–472.

Carr JA, Gentles A, Smith EE, Goleman WL, Urquidi LJ, Thuett K, et al. 2003. Response of larval *Xenopus laevis* to atrazine: assessment of growth, metamorphosis, and gonadal and laryngeal morphology. Environ Toxicol Chem 22:396–405.

Christin MS, Gendron AD, Brousseau P, Menard L, Marcogliese DJ, Cyr D, et al. 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. Environ Toxicol Chem 22:1127–1133.

Christin MS, Menard L, Gendron AD, Ruby S, Cyr D, Marcogliese DJ, et al. 2004. Effects of agricultural pesticides on the immune system of *Xenopus laevis* and *Rana pipiens*. Aquat Toxicol 67:33–43.

Ciba-Giegy Corporation. 1994. Environmental Fate Reference Data Source Book for Atrazine. Greensboro, NC:Ciba-Giegy Corporation.

Clements WH, Rohr JR. 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ Toxicol Chem 28:1789–1800.

Coady KK, Murphy MB, Villeneuve DL, Hecker M, Jones PD, Carr JA, et al. 2004. Effects of atrazine on metamorphosis, growth, and gonadal development in the green frog (*Rana clamitans*). J Toxicol Env Health A 67:941–957.

Cottingham KL, Lennon JT, Brown BL. 2005. Knowing when to draw the line: designing more informative ecological experiments. Front Ecol Environ 3:145–152.

Crain DA, Guillette LJ, Rooney AA, Pickford DB. 1997. Alterations in steroidogenesis in alligators (*Alligator mississippiensis*) exposed naturally and experimentally to environmental contaminants. Environ Health Perspect 105:528–533.

Das PC, McElroy WK, Cooper RL. 2001. Alteration of catecholamines in pheochromocytoma (pc12) cells *in vitro* by the metabolites of chlorotriazine herbicide. Toxicol Sci 59:127–137.

Davies PE, Cook LSJ, Goenarso D. 1994. Sublethal responses to pesticides of several species of Australian fresh-water fish and crustaceans and rainbow trout. Environ Toxicol Chem 13:1341–1354.

de Noyelles F, Kettle WD, Fromm CH, Moffett MF, Dewey SL. 1989. Use of experimental ponds to assess the effects of a pesticide on the aquatic environment. In: Using Mesocosms to Assess the Aquatic Ecological Risk of

Pesticides: Theory and Practice (Voshell JR, ed). Lanham, MD:Entomological Society of America, 41–56.

Diana SG, Resetarits WJ Jr, Schaeffer DJ, Beckmen KB, Beasley VR. 2000. Effects of atrazine on amphibian growth and survival in artificial aquatic communities. Environ Toxicol Chem 19:2961–2967.

Du Preez LH, Kunene N, Everson GJ, Carr JA, Giesy JP, Gross TS, et al. 2008. Reproduction, larval growth, and reproductive development in African clawed frogs (*Xenopus laevis*) exposed to atrazine. Chemosphere 71:546–552.

Du Preez LH, Solomon KR, Carr JA, Giesy JP, Gross TS, Kendall RJ, et al. 2005. Population structure of the African clawed frog (*Xenopus laevis*) in maize-growing areas with atrazine application versus non-maize-growing areas in South Africa. Afr J Herpetol 54:61–68.

Dunier M, Swicki AK. 1993. Effects of pesticides and other organic pollutants in the aquatic environment on immunity of fish: a review. Fish Shellfish Immun 3:423–438.

Edwards WM, Shipitalo MJ, Lal R, Owens LB. 1997. Rapid changes in concentration of herbicides in corn field surface depressions. J Soil Water Conserv 52:277–281.

Eggert C. 2004. Sex determination: the amphibian models. Reprod Nutr Dev 44:539–549.

Englund G, Sarnelle O, Cooper SD. 1999. The importance of data-selection criteria: meta-analyses of stream predation experiments. Ecology 80:1132–1141.

Evans JO, Duseja DR. 1973. Herbicide's Contamination of Surface Runoff Waters. EPA R2-73-266. Washington, DC:U.S. Environmental Protection Agency.

Fatima M, Mandiki SNM, Douxfils J, Silvestre F, Coppe P, Kestemont P. 2007. Combined effects of herbicides on biomarkers reflecting immune-endocrine interactions in goldfish immune and antioxidant effects. Aquat Toxicol 81:159–167.

Forson D, Storfer A. 2006a. Effects of atrazine and iridovirus infection on survival and life-history traits of the long-toed salamander (*Ambystoma macrodactylum*). Environ Toxicol Chem 25:168–173.

Forson DD, Storfer A. 2006b. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. Ecol Appl 16:2325–2332.

Frank R, Braun HE, Ripley BD, Clegg BS. 1990. Contamination of rural ponds with pesticide, 1971–85, Ontario, Canada. Bull Environ Contam Toxicol 44:401–409.

Freeman JL, Beccue N, Rayburn AL. 2005. Differential metamorphosis alters the endocrine response in anuran larvae exposed to T-3 and atrazine. Aquat Toxicol 75:263–276.

Freeman JL, Rayburn AL. 2005. Developmental impact of atrazine on metamorphing *Xenopus laevis* as revealed by nuclear analysis and morphology. Environ Toxicol Chem 24:1648–1653.

Gendron AD, Marcogliese DJ, Barbeau S, Chrsitin MS, Brousseau P, Ruby S, et al. 2003. Exposure of leopard frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. Oecologia 135:469–476.

Giddings JM, Anderson TA, Hall LW, Kendall RJ, Richards RP, Solomon KR, et al. 2005. A Probabilistic Aquatic Ecological Risk Assessment of Atrazine to North American Surface Waters. Pensacola, FL:SETAC Press.

Gurevitch J, Hedges LV. 1993. Meta-analysis: combining the results of independent experiments. In: The Design and Analysis of Ecological Experiments (Scheiner SM, Gurevitch J, eds). New York:Chapman & Hall, 378–398.

Hatfield JL, Wesley CK, Prueger JH, Pfeiffer RL. 1996. Herbicide and nitrate distribution in central Iowa rainfall. J Environ Qual 25:259–264.

Hayes TB. 1998. Sex determination and primary sex differentiation in amphibians: genetic and developmental mechanisms. J Exp Zool 281:373–399.

Hayes TB. 2004. There is no denying this: defusing the confusion about atrazine. Bioscience 54:1138–1149.

Hayes TB, Case P, Chui S, Chung D, Haeffele C, Haston K, et al. 2006. Pesticide mixtures, endocrine disruption, and amphibian declines: are we underestimating the impact? Environ Health Perspect 114:40–50.

Hayes TB, Collins A, Lee M, Mendoza M, Noriega N, Stuart AA, et al. 2002. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci USA 99:5476–5480.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A. 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ Health Perspect 111:568–575.

Hecker M, Giesy JP, Jones PD, Jooste AM, Carr JA, Solomon KR, et al. 2004. Plasma sex steroid concentrations and gonadal aromatase activities in African clawed frogs (*Xenopus laevis*) from South Africa. Environ Toxicol Chem 23:1996–2007.

Hecker M, Kim WJ, Park JW, Murphy MB, Villeneuve D, Coady KK, et al. 2005a. Plasma concentrations of estradiol and testosterone, gonadal aromatase activity and ultrastructure of the testis in *Xenopus laevis* exposed to estradiol or atrazine. Aquat Toxicol 72:383–396.

Hecker M, Park JW, Murphy MB, Jones PD, Solomon KR, Van Der Kraak G, et al. 2005b. Effects of atrazine on cyp19 gene expression and aromatase activity in testes and on plasma sex steroid concentrations of male African clawed frogs (*Xenopus laevis*). Toxicol Sci 86:273–280.

Hill AB. 1965. The environment and disease: association or causation? Proc R Soc Med 58:295–300.

Houck A, Sessions SK. 2006. Could atrazine affect the immune system of the frog, *Rana pipiens*? Bios 77:107–112.

Huber W. 1993. Ecotoxicological relevance of atrazine in aquatic systems. Environ Toxicol Chem 12:1865–1881.

Iwasawa H, Yamaguchi K. 1984. Ultrastructural-study of gonadal development in *Xenopus laevis*. Zool Sci 1:591–600.

Jooste AM, Du Preez LH, Carr JA, Giesy JP, Gross TS, Kendall RJ, et al. 2005. Gonadal development of larval male *Xenopus laevis* exposed to atrazine in outdoor microcosms. Environ Sci Technol 39:5255–5261.

Kadoum AM, Mock DE. 1978. Herbicide and insecticide residues in tailwater pits: water and pit bottom soil from irrigated corn and sorghum fields. J Agr Food Chem 26:45–50.

Kerby JL, Storfer A. 2009. Combined effects of atrazine and chloropyrifos on susceptibility of the tiger salamander to *Ambystoma tigrinum* virus. EcoHealth 6:91–98.

Kettle WD. 1982. Description and Analysis of Toxicant-Induced Responses of Aquatic Communities in Replicated Experimental Ponds [PhD dissertation]. Lawrence, KS:University of Kansas.

Kiely T, Donaldson D, Grube A. 2004. Pesticide industry sales and usage: 2000 and 2001 market estimates. Washington, DC:U.S. Environmental Protection Agency. Available: http://www.epa.gov/oppbead1/pestsales/01pestsales/market_estimates2001.pdf [accessed 19 November 2009].

Kiesecker JM. 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proc Natl Acad Sci USA 99:9900–9904.

Klaassen HE, Kadoum AM. 1979. Distribution and retention of atrazine and carbofuran in farm pond ecosystems. Arch Environ Contam Toxicol 8:345–353.

Kloas W, Lutz I, Springer T, Krueger H, Wolf J, Holden L, et al. 2009. Does atrazine influence larval development and sexual differentiation in *Xenopus laevis*? Toxicol Sci 107:376–384.

Knutson MG, Richardson WB, Reineke DM, Gray BR, Parmelee JR, Weick SE. 2004. Agricultural ponds support amphibian populations. Ecol Appl 14:669–684.

Kolpin DW, Sneck-Fahrer D, Hallberg GR, Libra RD. 1997. Temporal trends of selected agricultural chemicals in Iowa's groundwater, 1982–1995: are things getting better? J Environ Qual 26:1007–1017.

Koprivnikar J, Forbes MR, Baker RL. 2006. Effects of atrazine on cercarial longevity, activity, and infectivity. J Parasitol 92:306–311.

Langerveld AJ, Celestine R, Zaya R, Mihalko D, Ide CF. 2009. Chronic exposure to high levels of atrazine alters expression of genes that regulate immune and growth-related functions in developing *Xenopus laevis* tadpoles. Environ Res 109:379–389.

Lanzel S. 2008. Atrazine and Info-disruption: Does the Pesticide Atrazine Disrupt the Transfer of Chemical Information in the Terrestrial Salamander, *Plethodon shermani*? [Master's thesis]. Pittsburgh, PA:Dusquene University.

Larson DL, McDonald S, Fivizzani AJ, Newton WE, Hamilton SJ. 1998. Effects of the herbicide atrazine on *Ambystoma tigrinum* metamorphosis: duration, larval growth, and hormonal response. Phys Zool 71:671–679.

McCarthy ID, Fuiman LA. 2008. Growth and protein metabolism in red drum (*Sciaenops ocellatus*) larvae exposed to environmental levels of atrazine and malathion. Aquat Toxicol 88:220–229.

McCoy KA, Bortnick LJ, Campbell CM, Hamlin HJ, Guillette LJ, St Mary CM. 2008. Agriculture alters gonadal form and function in the toad *Bufo marinus*. Environ Health Perspect 116:1526–1532.

McCoy KA, Guillette LJ. In press. Endocrine disruptors. In: Amphibian Biology. Vol 8a. Conservation and Decline of Amphibians (Heatwole HF, ed). Chipping Norton, New South Wales, Australia:Surrey Beatty & Sons.

McDaniel TV, Martin PA, Struger J, Sherry J, Marvin CH, McMaster ME, et al. 2008. Potential endocrine disruption of sexual development in free ranging male northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) from areas of intensive row crop agriculture. Aquat Toxicol 88:230–242.

Moore A, Lower N. 2001. The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Comp Biochem Physiol B Biochem Mol Biol 129:269–276.

Moore A, Waring CP. 1998. Mechanistic effects of a triazine pesticide on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Pesticide Biochem Physiol 62:41–50.

Murphy MB, Hecker M, Coady KK, Tompsett AR, Higley EB, Jones PD, et al. 2006a. Plasma steroid hormone concentrations, aromatase activities and GSI in ranid frogs collected from agricultural and non-agricultural sites in Michigan (USA). Aquat Toxicol 77:153–166.

Murphy MB, Hecker M, Coady KK, Tompsett AR, Jones PD, Du Preez LH, et al. 2006b. Atrazine concentrations, gonadal gross morphology and histology in ranid frogs collected in Michigan agricultural areas. Aquat Toxicol 76:230–245.

Oka T, Tooi O, Mitsui N, Miyahara M, Ohnishi Y, Takase M, et al. 2008. Effect of atrazine on metamorphosis and sexual differentiation in *Xenopus laevis*. Aquat Toxicol 87:215–226.

Osenberg CW, Sarnelle O, Cooper SD, Holt RD. 1999. Resolving ecological questions through meta-analysis: goals, metrics, and models. Ecology 80:1105–1117.

Papaefthimiou C, Zafeiridou G, Topoglidi A, Chaleplis G, Zografou S, Theophilidis G. 2003. Triazines facilitate neurotransmitter release of synaptic terminals located in hearts of frog (*Rana ridibunda*) and honeybee (*Apis mellifera*) and in the ventral nerve cord of a beetle (*Tenebrio molitor*). Comp Biochem Physiol C Toxicol Pharmacol 135:315–330.

Raffel TR, Martin LB, Rohr JR. 2008. Parasites as predators: unifying natural enemy ecology. Trends Ecol Evol 23:610–618.

Raffel TR, Rohr JR, Kiesecker JM, Hudson PJ. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. Funct Ecol 20:819–828.

Reeder AL, Foley GL, Nichols DK, Hansen LG, Wikoff B, Faeh S, et al. 1998. Forms and prevalence of intersexuality and effects of environmental contaminants on sexuality in cricket frogs (*Acris crepitans*). Environ Health Perspect 106:261–266.

Renner R. 2004. Controversy clouds atrazine studies. Environ Sci Technol 38:107A–108A.

Rodriguez VM, Thiruchelvam M, Cory-Slechta DA. 2005. Sustained exposure to the widely used herbicide atrazine: altered function and loss of neurons in brain monoamine systems. Environ Health Perspect 113:708–715.

Rohr JR, Crumrine PW. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. Ecol Appl 15:1135–1147.

Rohr JR, Elskus AA, Shepherd BS, Crowley PH, McCarthy TM, Niedzwiecki JH, et al. 2003. Lethal and sublethal effects of atrazine, carbaryl, endosulfan, and octylphenol on the streamside salamander, *Ambystoma barbouri*. Environ Toxicol Chem 22:2385–2392.

Rohr JR, Elskus AA, Shepherd BS, Crowley PH, McCarthy TM, Niedzwiecki JH, et al. 2004. Multiple stressors and salamanders: effects of an herbicide, food limitation, and hydroperiod. Ecol Appl 14:1028–1040.

Rohr JR, Kerby JL, Sih A. 2006a. Community ecology as a framework for predicting contaminant effects. Trends Ecol Evol 21:606–613.

Rohr JR, Palmer BD. 2005. Aquatic herbicide exposure increases salamander desiccation risk eight months later in a terrestrial environment. Environ Toxicol Chem 24:1253–1258.

Rohr JR, Raffel TR, Romansic JM, McCallum H, Hudson PJ. 2008a. Evaluating the links between climate, disease spread, and amphibian declines. Proc Natl Acad Sci USA 105:17436–17441.

Rohr JR, Raffel TR, Sessions SK, Hudson PJ. 2008b. Understanding the net effects of pesticides on amphibian trematode infections. Ecol Appl 18:1743–1753.

Rohr JR, Sager T, Sesterhenn TM, Palmer BD. 2006b. Exposure, postexposure, and density-mediated effects of atrazine on amphibians: breaking down net effects into their parts. Environ Health Perspect 114:46–50.

Rohr JR, Schotthoefer AM, Raffel TR, Carrick HJ, Halstead N, Hoverman JT, et al. 2008c. Agrochemicals increase trematode infections in a declining amphibian species. Nature 455:1235–1239.

Rohr JR, Swan A, Raffel TR, Hudson PJ. 2009. Parasites, info-disruption, and the ecology of fear. Oecologia 159:447–454.

Rymuszka A, Siwicki AK, Sieroslawska A. 2007. Determination

of modulatory potential of atrazine on selected functions of immune cells isolated from rainbow trout (*Oncorhynchus mykiss*). Centr Eur J Immunol 32:97–100.

Saglio P, Trijasse S. 1998. Behavioral responses to atrazine and diuron in goldfish. Arch Environ Contam Toxicol 35:484–491.

Sanderson JT, Seinen W, Giesy JP, van den Berg M. 2000. 2-Chloro-*s*-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? Toxicol Sci 54:121–127.

Scholz S, Mayer I. 2008. Molecular biomarkers of endocrine disruption in small model fish. Mol Cell Endocrinol 293:57–70.

Scott DE. 1994. The effect of larval density on adult demographic traits in *Ambystoma opacum*. Ecology 75:1383–1396.

Skelly DK. 1994. Activity level and the susceptibility of anuran larvae to predation. Anim Behav 47:465–468.

Smith DC. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–350.

Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, et al. 1996. Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem 15:31–76.

Solomon KR, Carr JA, Du Preez LH, Giesy JP, Kendall RJ, Smith EE, et al. 2008. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Crit Rev Toxicol 38:721–772.

Storrs SI, Kiesecker JM. 2004. Survivorship patterns of larval amphibians exposed to low concentrations of atrazine. Environ Health Perspect 112:1054–1057.

Storrs SI, Semlitsch RD. 2008. Variation in somatic and ovarian development: predicting susceptibility of amphibians to estrogenic contaminants. Gen Comp Endocrinol 156:524–530.

Sullivan KB, Spence KM. 2003. Effects of sublethal concentrations of atrazine and nitrate on metamorphosis of the African clawed frog. Environ Toxicol Chem 22:627–635.

Tierney KB, Singh CR, Ross PS, Kennedy CJ. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquat Toxicol 81:55–64.

U.S. EPA. 2002. List of Contaminants and Their MCLs. EPA 816-F-02-013. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2003. Interim Reregistration Eligibility Decision for Atrazine. EPA-HQ-OPP-2003-0367. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2005. Draft Final Report on Multi-chemical Evaluation of the Short-term Reproduction Assay with the Fathead Minnow. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA. 2007. Preliminary Interpretation of the Ecological Significance of Atrazine Stream-water Concentrations Using a Statistically Designed Monitoring Program. EPA-HQ-OPP-2007-0934-0004. Washington, DC:U.S. Environmental Protection Agency.

U.S. EPA (U.S. Environmental Protection Agency). 2009a. GENEEC2 (GENeric Estimated Environmental Concentration). Available: http://www.epa.gov/oppefed1/models/water/index.htm#geneec2 [accessed 18 November 2009].

U.S. EPA (U.S. Environmental Protection Agency). 2009b. Program Evaluation Glossary. Available: http://www.epa.gov/evaluate/glossary/m-esd.htm [accessed 24 November 2009].

van Dijk HFG, Guicherit R. 1999. Atmospheric dispersion of current-use pesticides: a review of the evidence from monitoring studies. Water Air Soil Pollut 115:21–70.

Walsh AH, Ribelin WE. 1975. The pathology of pesticide poisoning. In: The Pathology of Fish (Ribelin WE, Migaki E, eds). Madison, WI:University of Wisconsin Press, 515–557.

Wilbur HM, Collins JP. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–1314.

Wilson K, Hardy ICW. 2002. Statistical analysis of sex ratios. In: Sex Ratios: Concepts and Research Methods (Hardy ICW, ed). New York:Cambridge University Press, 48–92.

Witschi E. 1929. Studies on sex differentiation and sex determination in amphibians. III. Rudimentary hermaphroditism and Y chromosome in *Rana temporaria*. J Exp Zool 54:157–223.

Zeeman MG, Brindley WA. 1981. Effects of toxic agents upon fish immune systems: a review. In: Immunologic Consideration in Toxicology (Sharma RP, ed). Boca Raton, FL:CRC Press, 1–47.

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:35 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 7:24 PM
To: EEPtestimony
Cc: jessicamitchell51@yahoo.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
JessicaMitchell	Individual	Support	Yes

Comments: Atrazine is banned in the European Union for a few years now.. We should be considering the same!!

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:34 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 4:57 PM
To: EEPtestimony
Cc: jjkauai@gmail.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
jonathan jay	Individual	Support	No

Comments: please support. mahalo! jj

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:35 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 8:21 PM
To: EEPtestimony
Cc: joy.shih@gmail.com
Subject: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

HR100
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Joy Leilei Shih	Individual	Support	Yes

Comments: Atrazine is an herbicide used in Hawaii and known to act an endocrine disruptor in all types of life, including amphibians, fish, and mammals. Its safety has not been established yet Hawaii ranks 10th in the nation in population exposed to atrazi ne according to a 2009 New York Times study. It has shown to cause demasculination, act as an estrogen disruptor, have carcinogenic effects, and is linked to epidemiological low sperm levels in men, thus having several researchers to call for banning it in the US. It is also a potential cause of birth defects, low birth weights and menstrual problems when consumed at concentrations below federal standards. Additional studies have tended to support the conclusion that even low doses can increase health risks, leading to calls for further testing and renewed EPA evaluation of atrazine's safety. The EU banned atrazine in 2004 due to the evidence showing harmful health effects. Atrazine's negative health effects all occur at levels currently deemed acceptable by the EPA. Due to the large amount of evidence and controversy surrounding Atrazine, Hawaii owes it to its people to asses its safety so that we can preserve our most precious resources, our water and our health, especially the health of our developing Keiki. Atrazine in Hawaii is not monitored sufficiently due to shortage in staff. The largest buyers of the chemical are Hawaiian seed corn companies Monsanto and Mycogen. Syngenta recently reached a class action settlement in City of Greeneville v. Syngenta Crop Protection, Inc., providing the Kaua'i Department of Water with $6,692.96 for atrazine clean-up expenses. Please perform your due diligence in protecting the health of our people by establishing a task force to study the safety of atrazine for people. Just the existence of this consideration is enough to support the need for this important measure. Mahalo for the opportunity to testify. A selection of references: "Chemicals in the News: Atrazine". Australian Pesticides and Veterinary Medicines Authority. 2010-06-30. Retrieved 2010-11-28. Beane Freeman, Laura E. (2011) Atrazine and Cancer Incidence Among Pesticide Applicators in the Agricultural Health Study (1994-2007). Environmental Health Perspetives. Jennifer Lee (2003-06-19). "Popular Pesticide Faulted for Frogs' Sexual Abnormalities". The New York Times. Tyrone Hayes, Kelly Haston, Mable Tsui, Anhthu Hoang, Cathryn Haeffele, and Aaron Vonk (2003). "Atrazine-Induced Hermaphroditism at 0.1 ppb in American Leopard Frogs" (Free full text). Environmental Health Perspectives 111 (4): 568. doi:10.1289/ehp.5932. Mizota, K.; Ueda, H. (2006).

"Endocrine Disrupting Chemical Atrazine Causes Degranulation through Gq/11 Protein-Coupled Neurosteroid Receptor in Mast Cells". Toxicological Sciences 90 (2): 362. doi:10.1093/toxsci/kfj087. PMID 16381660 "Pesticide atrazine can turn male frogs into females" (Press release). University of California. Retrieved March 5, 2010. Arbuckle, T.E., Z. Lin, and L.S. Mery, An exploratory analysis of the effect of pesticide exposure on the risk ofspontaneous abortion in an Ontraio farm population. Environ. Health Perspect., 2001. 109(8): p. 851-857. Swan, S., et al., Semen quality in relation to biomarkers of pesticide exposure. Environ. Health Perspect., 2003. 111(12): p. 1478-1484. Sanderson, J., et al., Induction and inhibition of aromatase (CYP19) activity by various classes of pesticides in H295R human adrenocortical carcinoma cells. Toxicol. Appl. Pharmacol., 2002. 182: p. 44-54. Sanderson, J.T., et al., Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ. Health Perspect., 2001. 109: p. 1027-1031. Heneweer, M., M. van den Berg, and J. Sanderson, A comparison of human H295R and rat R2C cell lines as in vitro screening tools for effects on aromatase. Toxicol. Letters, 2004. 146: p. 183-194. Sanderson, J.T., et al., 2-chloro-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: A novel mechanism for estrogenicity? Toxicol. Sci., 2000. 54: p. 121-127. Fan, W., et al., Atrazine-induced aromatase expression is SF-1- dependent: Implications for endocrine disruption in wildlife and reproductive cancers in humans. Environ. Health Perspect, 2007. doi:10.1289/ehp.9758 (available at http://dx.doi.org/). Roberge, M., H. Hakk, and G. Larsen, Atrazine is a competitive inhibitor of phosphodiesterase but does not affect the estrogen receptor. Toxicol. Letters, 2004. 154: p. 61-68. Maclennan, P., et al., Cancer incidence among triazine herbicide manufacturing workers. JOEM, 2002. 44(11): p. 1048-1058. Sass, J., Letter to the editor. JOEM, 2003. 45(4): p. 1-2. Kettles, M.A., et al., Triazine exposure and breast cancer incidence: An ecologic study of Kentucky counties. Environ. Health Perspect., 1997. 105(11): p. 1222-1227. http://www.novartisoncology.com/page/extended_adjuvantbreast_ therapy.jsp?usertrack.filter_applied=true&NovaId=2229644963416064359 Whalen, M., et al., Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. Chemico-Biological Interactions, 2003. 145(3): p. 311-319. Zeljezic, D., et al., Evaluation of DNA damage induced by atrazine and atrazine-based herbicide in human lymphocytes in vitro using a comet and DNA diffusion assay. Toxicol. In Vitro, 2006. 20(6): p. 923-935. Mizota, K. and H. Ueda, Endocrine disrupting chemical atrazine causes degranulation through G(q/11) protein-coupled neurosteroid receptor in mast cells. Toxicol. Sci., 2006. 90(2): p. 362-368. Hooghe, R., S. Devos, and E. Hooghe-Peters, Effects of selected herbicides on cytokine production in vitro. Life Sciences, 2000. 66(26): p. 2519-2525.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 9:55 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Thursday, March 28, 2013 9:42 AM
To: EEPtestimony
Cc: farmfreshhawaii@gmail.com
Subject: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

HR100
Submitted on: 3/28/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Juanita Brown Kawamoto	Individual	Support	Yes

Comments: All commercial farming activities or large scale farming programs need to participate in a un biased commmunity operated on-line register to provide disclosure to all communities in the State of Hawaii. Citizens Right to Know is what makes our communites safe and healthy. Please support this resolution. Mahalo for the opportunity to testify.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Wednesday, March 27, 2013 3:53 PM
To: HLTtestimony
Subject: FW: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 3:31 PM
To: EEPtestimony
Cc: judie@aloha.net
Subject: Submitted testimony for HR100 on Mar 28, 2013 10:00AM

HR100
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Judie Hoeppner	Individual	Support	No

Comments: I think it's important to have a study that will convince local and state politicians that there is indeed a danger to humans from exposure to Atrazine. The nature of the exposure i.e time, length and how needs to be clarified.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Wednesday, March 27, 2013 3:53 PM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 2:59 PM
To: EEPtestimony
Cc: karinmedigo@yahoo.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
karin medigovich	Individual	Support	No

Comments: We on the westside of Kauai need free public testing for urine and blood levels of pesticides such as Chlorpyrifos and Atrazine we have high disease rates in our neighborhoods and are exposed to many Agrocultural companies pesticides. please help us, thank you.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:34 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 5:44 PM
To: EEPtestimony
Cc: kawaiwarrenkhha@gmail.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
kawai Warren	Individual	Support	No

Comments: HCR 129 March 28, 2013 Conference room 325 State Capitol To: Director of Health I would like to request the Director of Health to establish a task force to study the effects of Atrazine on human health. Mahalo Kawai Warren Po Box 291 Kekaha Hawai'i 96752

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:35 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 6:40 PM
To: EEPtestimony
Cc: leslielarsen@earthlink.net
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Leslie Larsen	Individual	Support	No

Comments: Please - test the water so we can see if Atrazine is in it. For our Keiki.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:32 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 4:19 PM
To: EEPtestimony
Cc: Loralyne@earthlink.net
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Lora lynne	Individual	Support	No

Comments: This chemical herbicide needs to be out of use on these islands, now! Protect us all from the assault and violence against citizens by chemical company use of this on the fields of Kauai and other islands.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:35 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 6:11 PM
To: EEPtestimony
Cc: lynlie@hawaii.edu
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Lynlie Waiamau	Individual	Support	No

Comments: As I resident of Kaua'i and I am extremely concerned about the use of Atrazine by Syngenta, Pioneer and Dow who grow thousands of acres of genetically engineered corn and experimental field tests of other crops on this island. In the process, the y are spraying huge quantities of chemicals 7 out of 10 days. I would like to know if any of these chemicals, but specifically Atrazine, are getting into our water supply. I support investigating other impacts to the soil, ocean and nearby residential communities by way of chemically laden dust. The westside has always been known for its layer of red dirt that coats everything, from homes to cars to trees and plants. We need to know how that is impacting the health of people living there. I would've supported a bill with more teeth, that would require better regulation of the usage of Atrazine and other chemicals. I support this resolution as a first step in bringing answers to our communities that desperately need it.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

Margaret Sheen
PO Box 510165
Kealia, HI 96751

3/27/13

TESTIMONY

Testifier's name: Margaret Sheen
Committees to which the comments are directed: EEP/HLT
Date of Hearing: March 28, 2013
Time of Hearing: 10:00 AM
Measure number: HCR 129

Tyrone B. Hayes, PhD. is Professor of the Laboratory for Integrative Studies in Amphibian Biology in the Dept. of Integrative Biology at the University of California. His primary research focuses on the role of environmental factors, such as the chemical atrazine, on the growth and development in amphibians. He has published more than 40 papers, over 150 abstracts and given more than 300 talks on the subject of atrazine.

After 49 years of using atrazine at or above 80 million pounds per year, many target weed species have become atrazine-resistant. In fact, the number of documented atrazine-resistant "super" weeds number more than 80. No other herbicide has produced such dramatic effects on the evolution of weeds.

Endocrinology is the study of hormones. Hormones (endocrine substances) control growth, reproduction, metaboism, development, behavior, immune function, and stress, among other functions critical for life. Hormones are also important in many disease states including diabetes and cancer. Endocrine disruptors, such as atrazine, which interfere with hormone production and/or activity, can affect any of these processes.

Dr. Tyrone Hayes research indicates that atrazine has a harmful affect on our environment, amphibians, fish, and mammals, which includes humans.

Atrazine has been denied regulatory approval by the European Union and is, banned in Europe, even in Switzerland, the home of the manufacturer, Sygenta.

I am in support of the HCR129 asking for a study of Atrazine and the effects on human health.

Margaret Sheen

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:40 AM
To: HLTtestimony
Subject: FW: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Thursday, March 28, 2013 6:43 AM
To: EEPtestimony
Cc: matt_lopresti@yahoo.com
Subject: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

HCR129
Submitted on: 3/28/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Matthew LoPresti	Individual	Support	No

Comments:

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:37 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 9:53 PM
To: EEPtestimony
Cc: 2da1wahine@gmail.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Sandra Herndon	Individual	Support	No

Comments: Attn: EEP/HLT, FIN committes REQUESTING THE DIRECTOR OF HEALTH TO ESTABLISH A TASK FORCE TO STUDY THE EFFECTS OF ATRAZINE ON HUMAN HEALTH. Aloha! I request the Director of Health Dept to form a task force to INDEPENDENTLY study the effects of Atrazine on human health. It's already been detected in the water system of Waimea school on Kauai, and I fear that the children in this area are a great risk, as would be the elderly. Mahalo nui loa for your support.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:36 AM
To: HLTtestimony
Subject: FW: *Submitted testimony for HR100 on Mar 28, 2013 10:00AM*

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 9:13 PM
To: EEPtestimony
Cc: farmtokeiki@gmail.com
Subject: *Submitted testimony for HR100 on Mar 28, 2013 10:00AM*

HR100
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Tiana Kamen	Farm to Keiki	Support	No

Comments:

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Wednesday, March 27, 2013 3:54 PM
To: HLTtestimony
Subject: FW: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 3:33 PM
To: EEPtestimony
Cc: tomdee55@mac.com
Subject: *Submitted testimony for HCR129 on Mar 28, 2013 10:00AM*

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Tom DeCaro	Individual	Support	No

Comments:

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

morikawa2 - Shaun

From: thielen3 - Charles on behalf of EEPtestimony
Sent: Thursday, March 28, 2013 7:34 AM
To: HLTtestimony
Subject: FW: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

From: mailinglist@capitol.hawaii.gov [mailto:mailinglist@capitol.hawaii.gov]
Sent: Wednesday, March 27, 2013 5:37 PM
To: EEPtestimony
Cc: hawaiiannews@hawaii.rr.com
Subject: Submitted testimony for HCR129 on Mar 28, 2013 10:00AM

HCR129
Submitted on: 3/27/2013
Testimony for EEP/HLT on Mar 28, 2013 10:00AM in Conference Room 325

Submitted By	Organization	Testifier Position	Present at Hearing
Toni Auld Yardley	Individual	Support	No

Comments: Make HAWAI`I PROUD and PASS THIS RESOLUTION. Your Committee KNOWS - This is the PONO thing to do. Malama Pono. Aloha Aina. Hawai`i Pono`i. - Eo! Mahalonuiloa.

Please note that testimony submitted less than 24 hours prior to the hearing , improperly identified, or directed to the incorrect office, may not be posted online or distributed to the committee prior to the convening of the public hearing.

Do not reply to this email. This inbox is not monitored. For assistance please email webmaster@capitol.hawaii.gov

Aloha Ways and Means Chair and Committee Members.

I am a health care practice management consultant to professionals and do review studies frequently, although I am not a scientist. I am aware that the EPA is reviewing atrazine this year. Atrazine has been banned in Europe since 2004 and even in this country, but been was renewed in this country and is widely used as an herbicide as a weed killer. It has also come to my attention that because of the links between industry and the EPA, and the financial consequences to once again ban atrazine will have economic consequences to industry, but will most likely have a positive effect on human and animal health and cut the costs of health care due to the high levels of toxic burden from birth to the aged. I'm presenting some health studies here and information you may or may not be aware of.

New Research: Herbicide Atrazine Linked to Cancer, Birth Defects, Endocrine Disruption, and Endangered Species Impacts
http://www.biologicaldiversity.org/news/press_releases/2009/atrazine-08-27-2009.html

"Increase in Nutritionally Important Sweet Corn Kernel Carotenoids following Mesotrione and Atrazine Applications"
http://pubs.acs.org/doi/abs/10.1021/jf9013313 This article demonstrates what typically has occurred with industry funded studies on chemicals. That without actually studying humans, suppositions are made on their safety. In 2009, University of Tennessee Department of Plant Sciences researchers found the combination of the herbicides mesotrione and atrazine can make sweet corn more nutritious. They found the herbicides directly up-regulate the carotenoid biosynthetic pathway in corn kernels, which is associated with the nutritional quality of sweet corn. On reading the summary of the study, the researchers attempt to make an inference of human benefit that the herbicide induced in corn to dietary carotenoids thatfunction in suppressing aging eye diseases such as macular degeneration, now affecting 1.75 million older Americans. There

is no scientific proof to any claims of link in humans—particularly given that the biotech companies are not willing nor required to do human studies by the FDA, and with the only requirement to do 3 month rat studies before putting GMO/atrazine corn into the public food supply unlabeled, suggest no precaution has been taken. It's time.

Serious birth defects linked to the agricultural chemical atrazine
http://www.naturalnews.com/028222_atrazine_birth_defects.html

Atrazine associated with risk of small babies, human study shows
http://www.environmentalhealthnews.org/ehs/newscience/prebirth-atrazine-increases-risk-of-small-birth-size/

Is Common Pesticide responsible for rare birth defect?
http://www.safelawns.org/blog/2011/05/is-common-pesticide-responsible-for-rare-birth-defect/

Agency for Toxic Substancies and Disease Registry: Toxic Substances Portal- Atrazine http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=854&tid=59
Banning Atrazine Would Not Hurt Farmers
http://environment.about.com/od/healthenvironment/a/How-Dangerous-Is-Atrazine.htm

A study by the U.S. Department of Agriculture (USDA) suggests that if atrazine were banned in the United States, the drop in corn yields would be only about 1.19 percent, and the corn acreage would be reduced by only 2.35 percent. Dr. Frank Ackerman, an economist at Tufts University, concluded that estimates of higher corn losses were flawed due to problems in methodology. Ackerman found that despite a 1991 ban on atrazine in both Italy and Germany, neither country has recorded significant adverse economic effects.

In his report, Ackerman wrote there was "no sign of yields dropping in Germany or Italy after 1991, relative to the U.S. yield—as would be the case if atrazine were essential. Far from showing any slowdown after 1991, both Italy and (especially) German show faster growth in harvested areas after banning atrazine than before."

Based on this analysis, Ackerman concluded that if "the yield impact is on the order of 1%, as USDA estimated, or close to zero, as suggested by the newer evidence discussed here, then the economic consequences [of phasing out atrazine] become minimal."

Conversely, the economic costs of continuing to use atrazine—both in water treatment and public health costs—could be significant when compared to the relatively small economic losses of banning the chemical.

LIHU‘E – Kaua‘i’s drinking water is safe and free of the chemical herbicide atrazine, according to David Craddick, manager of the Kaua‘i Count… Read more

WAIMEA — More than 300 people gathered at Waimea Canyon Middle School Sunday afternoon for a presentation about the effects of Atrazine, a herbicide used in agricultural fields near Waimea — a town described by Hawaiian activist Walter Ritte as the “central battle ground” in a fight against biotech companies and genetically modified organisms.

One of the world’s most widely used and controversial herbicides, Atrazine — which is manufactured by Syngenta — has been banned in Europe since 2004 due to groundwater contamination risks. Studies have also suggested the chemical is associated with a number of health problems, including birth defects, low-birth weight and reproductive issues.

The event on Kaua‘i’s Westside featured keynote speaker Tyrone Hayes, an expert on Atrazine and a biology professor at the University of California at Berkeley. Other presenters included Ritte and Dr. Lorrin Pang, Maui District Health Officer for the state Department of Health.

“When I tell you what Atrazine does to frogs, you should remember that our hormones are so similar to frog hormones that our pregnancy hormone will make this frog lay eggs,” Hayes said. “So, if I tell you what Atrazine does to reproductive capabilities in this frog, you should be thinking, ‘What about me?’”

Hayes said Atrazine, first introduced in 1958 in the U.S., is used particularly on GMO and experimental corn, and historically on sugarcane in Hawai‘i and Florida. Each year, the U.S. uses 80 million pounds of Atrazine, he said.

For the last 15 years, he has been studying the effects of Atrazine on the African clawed frog. As part of his study, Hayes proposed that Atrazine turns on Aromatase, an enzyme which turns testosterone, a male hormone, into estrogen, a female hormone.

“If you are a male exposed to Atrazine, your testosterone goes away, so you’re demasculinized, or chemically castrated,” he said. “And you’re also feminized because you’re making estrogen, which you should not be doing as a male.”

Hayes said he discovered when frogs that have been injected with minimal amounts of Atrazine grow up, up to 10 percent of the males turn completely into females. He said those effects are produced when introducing the equivalent of 1/1,000 of a grain of salt in a gallon.

Hayes said a typical farmer often applies the chemical at levels that are 290 million times higher than what he uses in the laboratory.

“Men who apply Atrazine (in the field) have 24,000 times the Atrazine in their urine than we use in our laboratory to chemically castrate frogs and fish,” he said. “Think about that. One of these guys could pee in a bucket, I could dilute their urine 24,000 times and I could use the Atrazine in their urine to chemically castrate and make hermaphrodites out of 24,000 buckets of 30 tadpoles each.”

In a 2003 study, Hayes said Shauna Swan found that men who could not get their wives pregnant and had low sperm counts had significantly higher levels of Atrazine in their urine, a correlation he said can’t be ignored.

“We know that the sperm goes away when you give a fish Atrazine, when you give a frog Atrazine, when you give a reptile Atrazine, when you give a bird Atrazine, when you give a rat Atrazine,” he said. “Testosterone goes down and the sperm goes away and now this correlation says there’s an association in humans as well.”

Atrazine legacy

Hayes said his frogs trapped in a contaminated aquarium are no different than a human fetus trapped in a contaminated amniotic fluid inside the placenta.

“The placenta was not designed to keep out the 80,000 chemicals that we’ve invented, and studies now show that we are exposed to over 300 chemicals before we leave the womb,” he said.

And the Atrazine legacy apparently carries on for generations, according to Hayes.

He said studies with rats have shown that Atrazine causes prostate and mammary cancer, immune failure, neural damage in offspring, abortions, prostate disease in pups, impaired mammary development and impaired growth and development.

“This rat was never exposed to Atrazine; this rat was affected by Atrazine that its grandmother was exposed to,” he said. “This means that my daughter, that all of your daughters, that their granddaughters could be impacted by chemicals that we’re using today. This is not about you and me ... We’re talking about using chemicals today that your grandchildren’s grandchildren may be impacted by.”

Potentiation

Pang focused on the dangers of combining chemicals, which he said occurs regularly on biotech farms on Kaua'i's Westside.

"I knew (the companies) were using combinations, but until the lawyers came on board ... I didn't know they were using so many in combination," he said. "When you combine (drugs), it's considered a new drug until proven otherwise ... Same with pesticides."

Pang said some of the chemicals the companies are using might stay in the environment five days, but could remain in the human body for six months.

"So if they spray pesticide A today, and B two weeks from now, ... I think they're there together," he said.

Pang said the problem with chemicals like Atrazine is the potentiation (to enhance the effect of a drug or chemical), which has not been studied.

"It's worrisome to have Atrazine in your water, but what about the 50 (other) chemicals?" he asked. "Will they potentiate Atrazine? Will they potentiate each other?"

Pang said the margin of safety with chemicals like Atrazine means nothing without knowledge of the potentiation.

"I don't care what you think these 50 chemicals do or don't do, you've got to prove it by doing a little experiment, not telling me what you think," he said.

Westside vs. Pioneer

Honolulu-based attorney Gerard Jervis is representing the Westside community in a lawsuit against a seed company.

"As you know, Waimea residents have filed a lawsuit against ... Pioneer Hi-Bred International, Inc.," Jervis said at the meeting. "This meeting today is not about that lawsuit ... This is an informational meeting that is intended to educate the community about what's going on here. We believe that education is a powerful thing."

The location of Sunday's event seemed an appropriate fit, as Jervis said the U.S. Department of Agriculture tested the water in Waimea for Atrazine and "found positive results at this very middle school in the drinking fountains.http://thegardenisland.com/news/local/experts-atrazine-a-real-threat-to-westside-residents/article_bb8ba5bc-855b-11e2-85c9-001a4bcf887a.html

Natural Resources Defense Council http://www.nrdc.org/health/atrazine/
Atrazine Continues to Contaminate Surface Water and Drinking Water in the United States Approximately 75 percent of stream water and about 40 percent of all groundwater samples from agricultural areas tested in an extensive U.S. Geological Survey study contained atrazine. NRDC found that the U.S. EPA's inadequate monitoring systems and weak regulations have compounded the problem, allowing levels of atrazine in watersheds and drinking water to peak at extremely high concentrations.

The most recent data confirms that atrazine continues to contaminate watersheds and drinking water. Atrazine was found in 80 percent of drinking water samples taken in 153 public water systems. All twenty watersheds sampled in 2007 and 2008 had detectable levels of atrazine, and sixteen had average concentrations above the level that has been shown to harm plants and wildlife.

Wikipedia http://en.wikipedia.org/wiki/Atrazine

Atrazine

From Wikipedia, the free encyclopedia

Jump to: navigation, search

Atrazine

IUPAC name [hide]

1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine

Other names [hide]

Atrazine

Identifiers

CAS number	1912-24-9 Yes
PubChem	2256
ChemSpider	2169 Yes
UNII	QJA9M5H4IM Yes
DrugBank	DB07392
KEGG	C06551 Yes
ChEBI	CHEBI:15930 Yes
ChEMBL	CHEMBL15063 Yes
Jmol-3D images	Image 1
SMILES	
	[show]
InChI	
	[show]

Properties

Molecular formula	$C_8H_{14}ClN_5$
Molar mass	215.68 g mol^{-1}
Appearance	colorless solid
Density	1.187 g cm^{-3}
Melting point	

175 °C, 448 K, 347 °F

Boiling point

200 °C, 473 K, 392 °F
Solubility in water 7 mg/100 mL
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa)
Infobox references

Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine, an organic compound consisting of an s-triazine-ring is a widely used herbicide.

Its use is controversial due to widespread contamination of drinking water and its possible associations with birth defects and menstrual problems when consumed by humans at concentrations below government standards.[1] Although it has been banned in the European Union,[2] it is still one of the most widely used herbicides in the world.

Contents

Uses

Atrazine is used to stop pre- and post-emergence broadleaf and grassy weeds in major crops. The compound is both effective and inexpensive, and thus is well-suited to production systems with very narrow profit margins, as is often the case with maize (corn). Atrazine is the most widely used herbicide in conservation tillage systems, which are designed to prevent soil erosion.

Its effect on yields has been estimated from 6% to 1%, with 3-4%

being the conclusion of one review.[3] In another study looking at combined data from 236 university corn field trials from 1986‼ 2005, atrazine treatments showed an average of 5.7 bushels more per acre than alternative herbicide treatments.[4]

Chemistry and biochemistry

Atrazine is prepared from cyanuric chloride, which is treated sequentially with ethylamine and isopropyl amine. Like other triazine herbicides, atrazine functions by binding to the plastoquinone-binding protein in photosystem II, which animals lack. Plant death results from starvation and oxidative damage caused by breakdown in the electron transport process. Oxidative damage is accelerated at high light intensity.[5]

Atrazine was invented in 1958 in the Geigy laboratories as the second of a series of 1,3,5-triazines.[6]

Biodegradation

Atrazine degrades in soil primarily by the action of microbes. The half-life of atrazine in soil ranges from 13 to 261 days.[7] Atrazine biodegradation can occur by two known pathways:

Hydrolysis of the C-Cl bond, followed by the ethyl and isopropyl groups, catalyzed by the hydrolase enzymes called AtzA, AtzB, and AtzC. The end product of this process is cyanuric acid, itself unstable with respect to ammonia and carbon dioxide. The best characterized organisms that use this pathway are of Pseudomonas sp. strain ADP.

Dealkylation of the amino groups to give 2-chloro-4-hydroxy-6-amino-1,3,5-triazine, the degradation of which is unknown. This path also occurs in Pseudomonas species as well as a number of bacteria.[8][9]

Rates of biodegradation are affected by atrazine's low solubility, thus surfactants may increase the degradation rate. Though the two alkyl moieties readily support growth of certain microorganisms, the atrazine ring is a poor energy source due to the oxidized state of ring carbon. In fact, the most common pathway for atrazine degradation involves the intermediate, cyanuric acid, in which carbon is fully oxidized, thus the ring is primarily a nitrogen source for aerobic microorganisms. Atrazine may be catabolized as a carbon and nitrogen source in reducing environments, and some aerobic atrazine degraders have been shown to use the compound for growth under anoxia in the presence of nitrate as an electron acceptor,[10] a process referred to as a denitrification. When atrazine is used as a nitrogen source for bacterial growth, degradation may be regulated by the presence of alternative sources of nitrogen. In pure cultures of atrazine-degrading bacteria, as well as active soil communitites, atrazine ring nitrogen, but not carbon are assimilated into microbial biomass.[11] Low concentrations of glucose can decrease the bioavailability, whereas higher concentrations promote the catabolism of atrazine.[12]

The genes for enzymes AtzA-C have been found to be highly conserved in atrazine-degrading organisms worldwide. The prevalence of these genes could be due to the mass transfer of AtzA-C on a global scale. In Pseudomonas sp. ADP, the Atz genes are located noncontiguously on a plasmid with the genes for mercury catabolism. This plasmid is conjugatable to Gram-negative bacteria in the laboratory and could lead to the worldwide distribution, in view of the extensive release of atrazine and mercury. AtzA-C genes have also been found in a Gram-positive bacterium, but are chromosomally located.[13] The

insertion elements flanking each gene suggest that they are involved in the assembly of this specialized catabolic pathway.[9] Two options exist for degradation of atrazine using microbes, bioaugmentation or biostimulation.[9] Recent research suggests that microbial adaptation to atrazine has occurred in some fields where the herbicide is used repetitively, resulting in a decrease in herbicidal effectiveness.[14] Like the herbicides trifluralin and alachlor, atrazine is susceptible to rapid transformation in the presence of reduced iron-bearing soil clays, such as ferruginous smectites. In natural environments, some iron-bearing minerals are reduced by specific bacteria in the absence of oxygen, thus the abiotic transformation of herbicides by reduced minerals is viewed as "microbially induced".[15]

Health and environmental effects

According to Extension Toxicology Network in the U.S., "The oral median Lethal Dose or LD50 for atrazine is 3090 mg/kg in rats, 1750 mg/kg in mice, 750 mg/kg in rabbits, and 1000 mg/kg in hamsters. The dermal LD50 in rabbits is 7500 mg/kg and greater than 3000 mg/kg in rats. The 1-hour inhalation LC50 is greater than 0.7 mg/L in rats. The 4-hour inhalation LC50 is 5.2 mg/L in rats."[16]

Atrazine use in pounds per square mile by county. Atrazine is one of the most commonly used herbicides in the United States.[17]

Atrazine was banned in the European Union (EU) in 2004 because of its persistent groundwater contamination.[3] In the United States, however, atrazine is one of the most widely used herbicides, with 76 million pounds of it applied each year, in spite of the restriction that used to be imposed.[18][19] Its endocrine disruptor effects, possible

carcinogenic effect, and epidemiological connection to low sperm levels in men has led several researchers to call for banning it in the US.[3]

In August 2009, atrazine was prominently featured in the New York Times as a potential cause of birth defects, low birth weights and menstrual problems when consumed at concentrations below federal standards.[1] A Natural Resources Defense Council's Report on Atrazine suggested that the EPA is ignoring atrazine contamination in surface and drinking water in the central United States.[20] Findings from further studies released in early 2010 have tended to support the conclusion that even low doses can increase health risks, leading to calls for further testing and renewed EPA evaluation of atrazine's safety.[21]

Research results from the U.S. National Cancer Institute's Agricultural Health Study published in 2011 concluded that "there was no consistent evidence of an association between atrazine use and any cancer site." The study tracked 57,310 licensed pesticied applicators over 13 years.[22] EPA also determined in 2000 "that atrazine is not likely to cause cancer in humans."[23]

Note: It is unclear whether this is because the licensed pesticide applicators wore protective clothing and were extremely careful with its application in regards to themselves and not exposed by pesticide drift that unsuspecting persons not prepared in such a way.

Effect on amphibians

Atrazine is a suspected teratogen, causing demasculinization in male northern leopard frog even at low concentrations,[24][25] and an estrogen disruptor.[26] A 2010 study found that atrazine rendered 75 percent of male frogs sterile and turned one in 10 into females.[27] A 2002 study found that exposure to atrazine caused male tadpoles to

turn into hermaphrodites - frogs with both male and female sexual characteristics.[28] But another study, requested by EPA and funded by Syngenta, was unable to reproduce these results.[29]

Tyrone Hayes at the University of California notes that all of the studies that failed to conclude that atrazine caused hermaphroditism were plagued by poor experimental controls and were funded by Syngenta, one of the companies that produce the chemical.[30] The U.S. Environmental Protection Agency (EPA) and its independent Scientific Advisory Panel (SAP) examined all available studies on this topic ¶ including Hayes' work ¶ and concluded that there are "currently insufficient data" to determine if atrazine affects amphibian development. Hayes, formerly part of the SAP panel, resigned in 2000 to continue studies independently.[31] The EPA and its SAP made recommendations concerning proper study design needed for further investigation into this issue. As required by the EPA, Syngenta conducted two experiments under Good Laboratory Practices (GLP) and inspection by the EPA and German regulatory authorities. The paper concluded "These studies demonstrate that long-term exposure of larval X. laevis to atrazine at concentrations ranging from 0.01 to 100 microg/l does not affect growth, larval development, or sexual differentiation."[32] Another independent study in 2008 determined that "the failure of recent studies to find that atrazine feminizes X. laevis calls into question the herbicide's role in that decline." A report written in Environmental Science and Technology (May 15, 2008) cites the independent work of researchers in Japan, who were unable to replicate Hayes' work. "The scientists found no hermaphrodite frogs; no increase in aromatase as measured by aromatase mRNA

induction; and no increase in vitellogenin, another marker of feminization."[33]

A study published in 2007 examined the relative importance of environmentally relevant concentrations of atrazine on trematode cercariae versus tadpole defense against infection. The principal ˚nding of the present study was that susceptibility of wood frog tadpoles to infection by E. trivolvis is increased only when hosts are exposed to an atrazine concentration of 30 ng/L and not to 3 ng/L.[34]

A 2008 study reported that tadpoles developed deformed hearts and impaired kidneys and digestive systems when exposed to atrazine in their early stages of life. Tissue malformation may have been induced by ectopic programmed cell death, although a mechanism was not identified.[35]

In 2009, University of Tennessee Department of Plant Sciences researchers found the combination of the herbicides mesotrione and atrazine can make sweet corn more nutritious. They found the herbicides directly up-regulate the carotenoid biosynthetic pathway in corn kernels, which is associated with the nutritional quality of sweet corn. Enhanced accumulation of lutein and zeaxanthin is important because dietary carotenoids function in suppressing aging eye diseases such as macular degeneration, now affecting 1.75 million older Americans.[36]

In 2010 the Australian Pesticides and Veterinary Medicines Authority (APVMA), found the chemical safe to use:

> The conclusion of the APVMA at that time, based on advice from DEWHA, was that atrazine is unlikely to have an adverse impact on frogs at existing levels of exposure. This

advice was consistent with findings by the US EPA in 2007 (see below) that atrazine does not adversely affect amphibian gonadal development.[37]

Furthermore, the APVMA responded to Hayes' 2010 published paper,[38] by stating that his findings "do not provide sufficient evidence to justify a reconsideration of current regulations which are based on a very extensive dataset."[37]

A 2010 study conducted by the U.S. Geological Survey observed substantial adverse reproductive effects on fish from atrazine exposure at concentrations below the USEPA water-quality guideline.[39]

See also

- Pesticides in the United States - Atrazine
- Endocrine disruptor
- Simazine

References

- ^ a b Duhigg, Charles (August 22, 2009). "Debating How Much Weed Killer Is Safe in Your Water Glass". The New York Times. Retrieved 2009-09-10.
- ^ "Chemicals in the News: Atrazine". Australian Pesticides and Veterinary Medicines Authority. 2010-06-30. Retrieved 2010-11-28.
- ^ a b c Ackerman, Frank (2007). "The economics of atrazine". International Journal of Occupational and Environmental Health 13 (4): 437–445. PMID 18085057.
- ^ Fawcett, Richard S. (2008) Twenty Years of University Corn Yield Data: With and Without Atrazine. North Central Weed Science Society.
- ^ Appleby, Arnold P.; Müller, Franz; Carpy, Serge (2001). "Weed Control". Ullmann's Encyclopedia of Industrial Chemistry. doi:10.1002/14356007.a28_165. ISBN 3-527-30673-0.

^ Wolfgang Kr‰mer (2007). "Modern Crop Protection Compounds, Volume 1". Wiley-VCH.
^ Interim Reregistration Eligibility Decision for Atrazine, U.S. EPA, January, 2003.
^ Zeng Y, Sweeney CL, Stephens S, Kotharu P. (2004). Atrazine Pathway Map. Wackett LP. Biodegredation Database.
^ a b c Wackett, L. P.; Sadowsky, M. J.; Martinez, B.; Shapir, N. (January 2002). "Biodegradation of atrazine and related s-triazine compounds: from enzymes to field studies". Applied Microbiology and Biotechnology 58 (1): 39‼45. doi:10.1007/s00253-001-0862-y. PMID 11831474.
^ Crawford, J. J., G.K. Sims, R.L. Mulvaney, and M. Radosevich (1998). "Biodegradation of atrazine under denitrifying conditions". Appl. Microbiol. Biotechnol. 49 (5): 618‼623. doi:10.1007/s002530051223. PMID 9650260.
^ Bichat, F., G.K. Sims, and R.L. Mulvaney (1999). "Microbial utilization of heterocyclic nitrogen from atrazine". Soil Sci. Soc. Am. J. 63: 100‼110. doi:10.2136/sssaj1999.03615995006300010016x.
^ Ralebitso TK, Senior E, van Verseveld HW (2002). "Microbial aspects of atrazine degradation in natural environments". Biodegradation 13: 11‼19. doi:10.1023/A:1016329628618.
^ Cai B, Han Y, Liu B, Ren Y, Jiang S. (2003). "Isolation and characterization of an atrazine-degrading bacterium from industrial wastewater in China". Letters in Applied Microbiology 36 (5): 272‼276. doi:10.1046/j.1472-765X.2003.01307.x. PMID 12680937.
^ Krutz, L.J., D.L. Shaner, C. Accinelli, R.M. Zablotowicz, and W.B. Henry (2008). "Atrazine dissipation in s-triazine-adapted and non-adapted soil from Colorado and Mississippi: Implications of enhanced degradation on atrazine fate and transport parameters". Journal of

Environmental Quality 37 (3): 848‒857. doi:10.2134/jeq2007.0448. PMID 18453406.
^ Xu, J., J. W. Stucki, J. Wu, J. Kostka, and G. K. Sims (2001). "Fate of atrazine and alachlor in redox-treated ferruginous smectite". Environmental Toxicology & Chemistry 20 (12): 2717‒2724. doi:10.1002/etc.5620201210.
^ Pesticide Information Profile: Atrazine, Extension Toxicology Network (Cooperative Extension Offices of Cornell University, Oregon State University, the University of Idaho, and the University of California at Davis and the Institute for Environmental Toxicology, Michigan State University), June 1996.
^ USGS Pesticide Use Maps
^ Walsh, Edward (2003-02-01). "EPA Stops Short of Banning Herbicide". Washington Post. pp. A14. Retrieved 2007-04-27.
^ "Restricted Use Products (RUP) Report: Six Month Summary List". Environmental Protection Agency. Archived from the original on 11 January 2010. Retrieved 1 December 2009.
^ The New York Times http://graphics8.nytimes.com/packages/pdf/us/NRDC-Atrazine-report.pdf?scp=2&sq=atrazine&st=cse.
^ Amster, Randall. Silent Spring Has Sprung, Truthout, March 19, 2010.
^ Beane Freeman, Laura E. (2011) Atrazine and Cancer Incidence Among Pesticide Applicators in the Agricultural Health Study (1994-2007). Environmental Health Perspetives.
^ Interim Reregistration Eligibility Decision for Atrazine, U.S. EPA, January, 2003.
^ Jennifer Lee (2003-06-19). "Popular Pesticide Faulted for Frogs' Sexual Abnormalities". The New York Times.
^ Tyrone Hayes, Kelly Haston, Mable Tsui, Anhthu Hoang, Cathryn Haeffele, and Aaron Vonk (2003). "Atrazine-Induced Hermaphroditism at

0.1 ppb in American Leopard Frogs" (Free full text). Environmental Health Perspectives 111 (4): 568. doi:10.1289/ehp.5932.
^ Mizota, K.; Ueda, H. (2006). "Endocrine Disrupting Chemical Atrazine Causes Degranulation through Gq/11 Protein-Coupled Neurosteroid Receptor in Mast Cells". Toxicological Sciences 90 (2): 362. doi:10.1093/toxsci/kfj087. PMID 16381660.
^ "Pesticide atrazine can turn male frogs into females" (Press release). University of California. Retrieved March 5, 2010.
^ Briggs, Helen. (April 15, 2002), Pesticide 'causes frogs to change sex'. BBC News. Retrieved on 2007-10-16.
^ Jooste et al.; Du Preez, LH; Carr, JA; Giesy, JP; Gross, TS; Kendall, RJ; Smith, EE; Van Der Kraak, GL et al. (2005). "Gonadal Development of Larval Male Xenopus laevis Exposed to Atrazine in Outdoor Microcosms". Environ. Sci. Technol. 39 (14): 5255‖5261. doi:10.1021/es048134q. PMID 16082954.
^ Hayes, TB (2004). "There Is No Denying This: Defusing the Confusion about Atrazine". BioScience 54 (12): 1138‖1149. doi:10.1641/0006-3568(2004)054[1138:TINDTD]2.0.CO;2. ISSN 0006-3568.
^ Weedkiller 'threatens frogs', BBC News. 31 October 2002
^ Kloas, W; Lutz, I; Springer, T; Krueger, H; Wolf, J; Holden, L; Hosmer, A (2009). "Does atrazine influence larval development and sexual differentiation in Xenopus laevis?". Toxicological sciences : an official journal of the Society of Toxicology 107 (2): 376‖84. doi:10.1093/toxsci/kfn232. PMC 2639758. PMID 19008211.
^ Atrazine effects in Xenopus aren't reproducible, Aquatic Toxicology
^ Koprivnikar, Janet; Forbes, Mark R.; Baker, Robert L. (2007). "Contaminant Effects on Host ‖ Parasite Interactions: Atrazine,

Frogs, and Trematodes". Environmental Toxicology and Chemistry 26 (10): 2166‒70. doi:10.1897/07-220.1. PMID 17867892.
^ Early Exposure To Common Weed Killer Impairs Amphibian Development
^ Kopsell, Dean A.; Armel, Gregory R.; Mueller, Thomas C.; Sams, Carl E.; Deyton, Dennis E.; McElroy, J. Scott; Kopsell, David E. (2009). "Increase in Nutritionally Important Sweet Corn Kernel Carotenoids following Mesotrione and Atrazine Applications". Journal of Agricultural and Food Chemistry 57 (14): 6362‒8. doi:10.1021/jf9013313. PMID 19537793.
^ a b 'Chemicals in the News: Atrazine', Australian Pesticides and Veterinary Medicines Authority, June 30, 2010
^ Hayes, TB; Khoury, V; Narayan, A; Nazir, M; Park, A; Brown, T; Adame, L; Chan, E et al. (2010). "Atrazine induces complete feminization and chemical castration in male African clawed frogs (Xenopus laevis)". Proceedings of the National Academy of Sciences of the United States of America 107 (10): 4612‒7. doi:10.1073/pnas.0909519107. PMC 2842049. PMID 20194757.
^ Commonly Used Atrazine Herbicide Adversely Affects Fish Reproduction, ScienceDaily (May 20, 2010)

Further reading

Fan W, Yanase T, Morinaga H, et al. (May 2007). "Atrazine-induced aromatase expression is SF-1 dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans". Environ. Health Perspect. 115 (5): 720‒7. doi:10.1289/ehp.9758. PMC 1867956. PMID 17520059.
Randall Amster, Silent Spring Has Sprung, Truthout, March 19, 2010.
Synonyms and brand names
Rohr, JR; McCoy K (Sept 23, 2009). "A Qualitative Meta-Analysis

Reveals Consistent Effects of Atrazine on Freshwater Fish and Amphibians". Environmental Health Perspectives 118 (1): 20‼ 32. doi:10.1289/ehp.0901164. PMC 2831963. PMID 20056568.
"The Triazine Herbicides" Published February 2008, Elsevier, ISBN 978-0-444-51167-6
Pat Thomas "Sex, lies and herbicides". The Ecologist. February/March 2006.

External links

- Atrazinelovers: an anti-atrazine website - maintained by Tyrone Hayes
- Atrazine News - an Atrazine specific news site
- atrazine.com - Syngenta's page about atrazine
- Saving the Oasis Atrazine response to Last Call at the Oasis movie - Syngenta website