STATISTICAL APPROACH FOR ASSESSING AQUATIC ATRAZINE CONCENTRATIONS AND POTENTIAL IMPACTS ON AMPHIBIANS

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ABSTRACT

Aquatic contaminant assessments using water quality data can be challenged if sampling frequency is less than daily, if sampling only happens during certain months of the year, and if sampling lacks appropriate adjustments for averaging partial or insufficient data. Random or long-interval data collection will miss temporary spikes in contaminant concentrations. This leads to underestimates of harmful chemical concentrations, which in turn affects risk assessment, hazard assessment, and further conclusions made from quantitative data. A year-round, once daily sampling scheme paired with temporal adjustment factors is proposed to accurately assess the impact on amphibians. The daily sampling technique has potential additional uses for assessing the impact of other environmental contaminants on amphibians, which are particularly susceptible to aquatic chemical contaminants due to their permeable skin and aquatic developmental phases of life. In this paper, the herbicide atrazine and its presence in fresh waterways is explored in relation to current standardized aquatic sampling techniques, as well as the need to make changes in sampling that could be applied in the field, directly impacting assessments relating to amphibians.

BIOGRAPHICAL SKETCH

Born in Maryland, Emma Skekel completed her Bachelor of Science degree at St. Mary's College of Maryland with a chemistry major and environmental studies minor. She obtained her degree with American Chemical Society (ACS) certification, signifying that she had met ACS requirements for professional training. Her future plans include a career in industry with environmental remediation applications.

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TABLE OF CONTENTS

Introduction

Herbicides in the Environment – Atrazine

The herbicide atrazine is an environmental contaminant that is widely used in the United States to kill grasses and broad-leaf weeds. Atrazine is a nitrogen-based compound and has been used in the U.S. since 1958 when it was first registered in the triazine family of herbicides, one of the most widely used classes of agricultural chemicals (Figure 1).[1] Atrazine is commonly used to kill weeds found in fields of corn, sugar cane, and sorghum crops; however, its uses extend from agricultural land to residential lawns.[2] The use of atrazine is so widespread because it is cost effective for weed control, but research conducted throughout the years has uncovered numerous negative side effects.

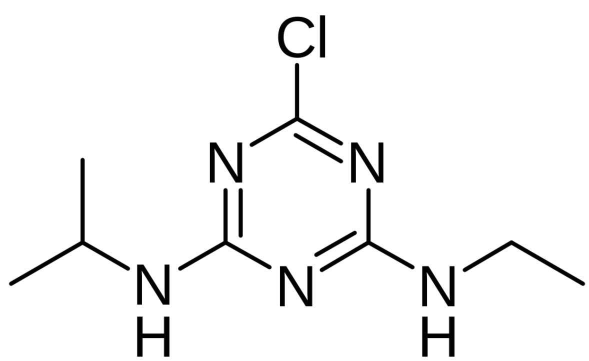

Figure 1. Chemical structure of atrazine. [3]

The triazine family of herbicides, and specifically atrazine, is one of the most extensively researched herbicide classes today.[2] This research has led some countries, specifically those in the European Union (EU), to ban atrazine in early 2004. The U.S. has not yet followed suit in banning the use of atrazine, but public concern and suggested health and ecological effects could cause a change in policy. The EU ban of atrazine was put into effect because of research performed on groundwater atrazine levels and the potential bodily effects that atrazine contamination could cause.[4] Atrazine was found in groundwater and drinking water at levels surpassing the 0.1 μg/L limit set by EU regulatory committees and was thought to pose harmful effects to humans if continued use occurred, though, the EU may have acted prematurely in banning atrazine. Some in the global community think that the atrazine concentration limit of 0.1 μg/L may have been set somewhat arbitrarily. The EU applied this limit not only to atrazine, but to all herbicides and pesticides. Little-to-no research was performed regarding atrazine prior to the EU limit being set, suggesting that the standard was not scientifically supported or health-based. Later on, researchers found that atrazine has the ability to harm endocrine systems, reproductive systems, and could even be linked to cancer in amphibians, which launched further studies to preserve the already-threatened species. [4-7]

Applying chemical compounds in nature to prevent or control invasive plants or insects has a history of adverse effects on environmental health and organisms. For example, dichloro-diphenyl-trichloroethane (DDT) was one of the first modern synthetic pesticides used to combat disease-carrying insects. DDT was banned in the U.S. due to its harmful effects on the environment, specifically for being an endocrine disruptor in the wildlife that came in contact with it, similar to the effects seen from atrazine on amphibians. Both DDT and atrazine have shown teratogenic effects on wildlife – DDT on birds and atrazine on frogs. DDT was banned before mounting scientific evidence was able to connect its damaging reproductive effects to both animals and humans, which is why DDT studies and data analysis continue to this day. However, unlike DDT, atrazine does not have widespread public concern urging the U.S. government to ban its use.[8] Scientific studies on atrazine where findings suggest that male tadpoles develop into female frogs within their 1-12 month metamorphosis (depending on the species of frogs) have data and conclusions similar to DDT studies, in that both chemicals show negative impacts on nature, leading researchers to perform lab testing that yields further teratogenic effects on organisms. These similarities suggest that the U.S. continues to risk atrazine's toxic and adverse effects on environmental and amphibian health for the sake of accessibility and economics.[9]

Effects on Amphibians

Herbicides such as atrazine may be effective at killing target plant species, but they often have ill-effects on non-targeted organisms residing near application sites. Atrazine has proven to be an endocrine disruptor, seen most often in amphibians and their reproductive systems, even at low levels of exposure (0.1 μg/L).[7, 10] Male frogs have been shown to develop female sex characteristics, specifically in the growth of eggs in the frog's testes. Amphibian offspring exposed to atrazine have also developed long term behavioral problems, increased susceptibility to infection, and even a shorter lifespan.[11] The harmful effects of atrazine exposure to amphibians have been researched, but information about atrazine's effects on aquatic life is still emerging. The endocrine disruptor screening program (EDSP) chose *Xenopus laevis* as the ideal organism for testing endocrine disrupting chemicals, with many researchers testing acute, high doses of atrazine and inferring chronic, long-term effects due to time constraints.[12, 13, 14]

Developing organisms are generally more susceptible to environmental hazards than their fully-grown counterparts, as is seen in bird eggs previously exposed to DDT and tadpoles exposed to atrazine. Amphibians have a complex life cycle and permeable skin that makes them particularly susceptible to chemicals such as atrazine, both on land and in water. With their two-staged life cycle (aquatic larvae and terrestrial adults), they are sensitive to atrazine applied in both environments. Even at the low concentration of 0.1 μg/L, *Xenopus laevis* tadpoles experienced testicular degeneration and impaired development of antiviral defenses.[13, 15]

Similarly, *Silurana tropicalis* tadpoles experienced spinal curvature and thyroid reduction after exposure to low doses of atrazine in aquatic environments.[16]

Amphibians are often underrepresented in risk assessment considerations, despite frequent exposure and susceptibility. Mammals are usually well-represented in risk assessment, but amphibian dermis is much more permeable to herbicides and can accumulate these harmful chemicals from aquatic habitats.[17] The amphibian population of the world has been declining for years now, especially in the western United States where chemical agricultural practices are extensive.[18] Amphibians are often thought to be an early warning signal for toxic environmental hazards due to their sensitive skin. Therefore, amphibians can show the adverse effects of herbicides well in advance of discovering toxicity in humans and other mammals, thus providing a chance to alter practices. In this case, altering practices could mean that an authoritative scientific organization or an appropriate U.S. government agency provides an improved and consistent sampling methodology. Additionally, a recommendation may be made ahead of long-time sampling and studies to discontinue the use of atrazine.

Amphibian exposure to peak concentrations of atrazine over a matter of hours poses great risk to a species with such porous skin. A saturated aqueous solution of atrazine has a concentration of about 35 mg/L at 22 degrees Celsius, with a higher likelihood of penetrating the skin if exposure lasts more than mere minutes.[19] [20] Low solubility herbicides, like atrazine, are more likely to adhere to soil particles and, therefore, will not travel very much with water as it moves. This causes atrazine to be present not only in water, but in sediment, both of which amphibians often make habitats due to their preference for shallow waters.[21] Through water and sediment contact, amphibians can be exposed to harmful amounts of atrazine in only a matter of hours, making it imperative that maximum concentration values are accounted for when measuring averages.

Current Sampling Methodology

Water quality testing techniques usually collect samples at regular intervals such as weekly, biweekly, or several times per month (Table I).[22] Monitoring herbicides used on agricultural fields can provide insight into ecosystem health, wildlife wellbeing, and groundwater contamination. Water quality data is often used to estimate quantities of interest in monitoring assessments, and can lead to changes in policy or health hazards. For example, the Environmental Protection Agency (EPA) generally does not require water utilities to notify its residents unless the yearly average atrazine concentration exceeds 3 μg/L. The desire to avoid public upset and financial responsibility gives incentive for government agencies to keep atrazine averages artificially low, and increases the importance for atrazine sampling methodologies to be as accurate as possible.

Table I. Sampling timescales commonly used for herbicide and pesticide analysis. Adapted from Charles G. Crawford, 2004.[22]

Sampling Timescale	Samples Per Year	Maximum Samples Per Month*
Quarterly	4	<1
1 sample in first and last quarter, 1 sample each in April and August	4	1
1 sample in first and last quarter, 1 sample each in April and August, twice monthly sampling in May, June, and July	10	2
Monthly	12	1
1 sample in first and last quarter, twice monthly sampling in April through August	12	2

1 sample in first and last quarter, twice monthly sampling in April and September, 4 times monthly sampling in May through August	22	4
Twice monthly	24	2
1 sample in first and last quarter, twice monthly sampling in April and September, 10 times monthly sampling in May through July	36	10
4 times monthly	48	4
10 times monthly	120	10

Note: *during herbicide/pesticide runoff interval.

A commonly raised question in herbicide measurement techniques is how likely peak concentrations may be missed if sampling intervals are less than daily. While hourly or even minutely concentrations would be more ideal than daily measurements, the most feasible option is daily sampling. Hourly sampling requires more analytical testing and with that comes time, manpower, and higher cost. An hourly measurement technique has the potential to create thousands of samples within a given time period that would require classification, analysis, and proper handling, which in turn creates unrealistic expectations on both the researchers and analytical chemists. For these reasons, hourly sampling has been foregone and daily sampling is suggested in the proposed methodology of this paper. Daily sampling can fit within financial and manpower constraints, while also proving much more statistically accurate data than, say, weekly sampling. The maxima that a daily sampling regime would account for are not necessarily valuable on their own, but are significant to the average chemical concentration(s) being measured. Similarly, researchers must ask to what extent this missed data may impact percentile estimates and rolling averages in an annual atrazine concentration profile. Unlike common data measurements such as means and medians, maxima are more difficult to estimate since they are highly variable both spatially and temporally and are more sensitive to watershed exposure and timing of atrazine use with weather events that impact pesticide runoff. For these reasons, a streamlined and reproducible sampling methodology that accounts for extremes is of practical interest in the water quality assessment field.

The EPA has previously proposed a method of statistical manipulation called bias factors to adjust atrazine sampling measurement data that was sampled at various frequencies.[23] Depending on the sample design and objective, the bias factor is multiplied by the sample objective to acquire a corrected and protective estimate of chemical concentration(s). The concept of bias factors has been proposed for atrazine, as well as other herbicides, but it remains unclear and requires more evaluation before becoming a useful tool in data analysis and sampling methodology. Bias factors serve as a protective aspect to be used in data assessment and policy/decision making regarding environmental atrazine concentrations and could be used when creating and evaluating future cumulative risk assessments of atrazine. These factors attempt to correct for underestimates in average concentrations over a given time interval, thus protecting against potential missed maximums. Despite lingering questions about the role, presentation, and application of bias factors, the initiative behind them is important and brings awareness to the ongoing issue of atrazine overuse and contamination in the environment. This paper aims to assess current atrazine sampling methodology and provide suggestions for an improved sampling method, evaluate its potential performance and expected benefits, relate it to aquatic life, and compare it to other sampling methods that have been successful and consistent in revealing herbicidal concentrations in water.

Modified Methods

Daily Water Sampling

With its low solubility in water and high mobility in soil, atrazine is a prime candidate for environmental contamination and buildup. The low solubility in water makes atrazine susceptible to runoff during weather events and its mobility makes it easy to enter into surface water by adhering to soil particles and washing into waterways. This causes large annual variations in atrazine contamination because when rain events occur, especially after new atrazine application, there is sizeable runoff. Atrazine sometimes enters waterways without mixing to form a saturated solution, thus occasionally requiring surfactants for removal. To gather water quality data that more accurately reflects an average atrazine concentration for a given location, researchers should be sampling daily. Gathering daily data to form an average prevents the researcher from missing any potential maxima that could negatively affect aquatic wildlife or build up in the environment.

To obtain useful measurements, scientists need to collect data for the target location with a likelihood of herbicide occurrence, in a timeframe in which herbicides are expected to be used, and where sampling is frequent enough to estimate exposures for the endpoints of concern.[23] Atrazine concentrations should be measured daily in the same location(s) and using proper protocol (i.e., use clean glassware, refrigerate sample, prevent contaminates from entering sample containers) in order to gather data that is most representative of a specific surface water system. Collecting daily concentrations provides data analysts with significantly more data than are usually collected, thus creating a larger sample size and allowing more accurate parameter estimates for a given location to be provided for risk assessments regarding environmental contaminants such as atrazine.

Statistically accurate parameter measurements are usually a goal when performing a study of any kind. The more accurate the parameter measurements, the more reliable conclusions can be drawn. This same concept should be applied when monitoring atrazine concentrations in surface water. The EPA has recently claimed that "monitoring data will typically underestimate actual exposure concentrations due to insufficient sampling frequency," (EPA, 2014) likely because maximums are missing from the collected data.[23]

Temporal Adjustment Factors

The temporal adjustment factor is an estimate that is bias-corrective and protective. Its product with a yearly sample estimate exceeds the true value of the yearly sample target quantity in 95% of all sample possibilities, as shown in the following probability statement:

(1) $$P(F * \phi > \Phi) = 0.95$$

where ϕ is the sample estimate, F is the temporal adjustment factor, Φ is the true value, and the probability is with respect to the sampling distribution of ϕ. The sample estimate can represent the yearly mean, 95^{th} percentile, or the maximum monthly rolling average. The distribution is the probability distribution of the sample estimator. For example, if sampling were every 14 days with a random start date, the method would provide 14 possible samples, so 14 possible estimates ϕ, each having a 1/14 probability of occurring.

Equation 1 above is an upper 95% confidence interval with the temporal adjustment factor as the unknown value, F, that when multiplied by ϕ provides the upper interval limit. In reference to a sampling distribution, the temporal adjustment factor is the true value divided by a constant k, obtained from the sampling distribution.

(2) $$F = \phi / k$$

The constant k equals the lower 5^{th} percentile for a continuous sampling distribution, such as those of atrazine measurements.

Calculating temporal adjustment factors requires knowing both ϕ and Φ, which involves knowing all population values (i.e., a concentration value for every day during the time period of interest). There will be a different temporal adjustment factor for every combination of data parameters, such as site-year, sampling frequency, and target value. In order to find a temporal adjustment factor that can be used for a data set that does not have daily or near-daily measurements, a reference temporal adjustment factor, F_{ref}, can be used.

Example: Suppose the target value is the maximum from a population of 25 (e.g. [1, …,25]) that is sampled once with each value having equal probability of being chosen. This gives a Φ of 25. The temporal adjustment factor would then be the following:

$$F = \Phi / k = 25 / k$$

Where *k* equals any value in the interval [1, 2), giving a 96% probability of the resulting interval containing the value 25. If *k* can be any value in the interval [1,2), then the temporal adjustment factor can be any value in the interval (12.5, 25].

To begin calculating F_{ref} using atrazine concentration data, a data set with daily measurements must first be identified. An ideal data set should be similar in characteristics to those where the F_{ref} will be applied. For each time period of interest in the data set, calculate the desired target quantity for all possible samples under the sampling design. Next, apply equation 1 to find the temporal adjustment factor. Note that the general approach (both in sampling design and quantile estimation) must match the general approach for which F_{ref} will be applied. Lastly, find the value of F_{ref}. The mean ($F_{ref(mean)}$) or median ($F_{ref(med)}$) of the reference set temporal adjustment factors can be used.

The product of F_{ref} and a target value estimate from the sample (not from the population) gives the adjusted estimate for that particular sample. Note that each sample produced by the sample design will generate a difference temporal adjustment factor final estimate. This approach differs from the EPA's proposed bias factors because of the calculated reference sets of temporal adjustment factors at chosen sampling frequencies. The EPA forgoes this step and instead calculates reference sets of bias factors at different sampling frequencies, then applies a regression equation to the data with independent variable sampling frequency.[23] The proposed temporal adjustment factor method instead calculates values directly at the desired sampling frequencies.

Calculating a temporal adjustment factor from a large population data set and applying that constant value to smaller sample data sets, assuming comparative sampling plans have similar characteristics and designs, creates a more accurate pool of data from which scientists can draw conclusions. After obtaining a single data set that has sampled daily or near-daily at a particular location, further sample designs are not required to measure daily if using the statistical adjustment. The temporal adjustment factor allows scientists to sample weekly, biweekly, monthly, etc. with statistical adjustment via F_{ref} taking place after data has been gathered. This method could prove to be more cost effective, time efficient, and simpler than current unregulated methods, and could be used as a streamlined method of sampling for atrazine from this time forward if daily sampling is not possible.

Discussion

The proposed statistical evaluation method of daily sampling and temporal adjustment factors could serve as critical tools when examining amphibians in nature. Designing and utilizing studies that give consistent data can prove difficult when there is no widely accepted methodology for aquatic atrazine analysis. While the EPA and USGS, along with several morefocused programs and agencies, have suggested multiple and varied "standards" for herbicide concentration measurement in waterways, there are still spatial and temporal discrepancies between publications.[18, 23, 24] Using the methodology described in this paper would allow researchers to complete one in-depth daily sampling of atrazine prior to more spread-out sampling timescales while also analyzing amphibian behavioral and physical characteristics and quantities. For example, if researchers planned on analyzing atrazine effects on local amphibians in and around a local pond, employing temporal adjustment factors could make research simpler and more efficient. Measuring daily atrazine concentrations at select points within the pond would provide a baseline for which F_{ref} could be evaluated. After F_{ref} values are obtained for one year at a specific site, any consecutive years and measurements could be made at less frequent intervals on a larger timescale without underestimating the atrazine concentration to which amphibians are exposed, assuming comparative sampling plans have similar characteristics and designs.

Adjusting sample data to represent a more accurate atrazine concentration in water systems of interest serves to both correct and protect data from outliers. Protecting data from outliers prevents underestimates of atrazine concentrations in waterways that serve as habitats for wildlife. Using temporal adjustments factors would allow scientists to design and implement one daily-measurement experiment to calculate appropriate F_{ref} values, then use those values in subsequent weekly, biweekly, or monthly sampling systems. This requires less work in the long run and provides more accurate atrazine concentration estimates for waterways that could potentially assist in environmental policymaking along with resultant organism health.

As mentioned previously, recent studies have found that amphibians are particularly susceptible to pesticides such as atrazine in their local aquatic environments. The vast majority of these studies have used a select few breeds of frogs and exposed them to regimented systems of acute atrazine doses to equate the effects of a more chronic exposure. While this method is acceptable for initial conclusions, a more in-depth analysis may require studies to observe and sample amphibians in their natural habitats.

Amphibians, particularly frogs, spend the majority of their aquatic stage in waterways during the spring season when they are either reproducing or still in their gill-breathing larvae stage.[25] Freshwater ponds and other shallow/still waters are preferred, with frogs generally choosing close-proximity waterways as their habitats. Due to this specific choice of habitat, frogs that choose atrazine-contaminated waterways could be exposed to higher levels of atrazine than moving bodies of water such as rivers or streams. Atrazine can only be removed from water via hydrolysis or bio-degradation, with the addition of humic material causing an increase in the rate of degradation via hydrolysis. Due to the preference of still, freshwater waterways, frogs are at greater risk for atrazine accumulation, making it essential to use a standard measurement method to ensure accuracy of atrazine concentrations to which amphibians are exposed.

Viewing the measuring of atrazine more broadly, the improved-accuracy data could be applied to national environmental decision-making. The methodology described in this paper could be used in lieu of or in addition to modeled estimates, and potentially even used as ancillary data with similar design objectives, and then applied directly for risk assessment. This could take place on a national scale, as well as on a regional or local scale, to suggest refinement and modifications to either current or nonexistent regulations on atrazine. The EPA has acknowledged that well-designed water monitoring studies must include a relevant temporal component in relation to monitoring if the data will be used quantitatively, providing even more incentive for temporal adjustment factors, bias factors, and other statistical correction methods to be employed when analyzing natural waterways.[23]

Application to Other Herbicides

While daily sampling and temporal adjustment factors have been discussed in this paper as an approach for aquatic atrazine concentration measurements, it also has potential for use with other herbicides. Atrazine has high mobility in soil and low solubility in water, thus giving it unique characteristics that pose greater risk for environmental accumulation. These chemical characteristics of atrazine are not present in all herbicides; many behave differently in terrestrial and aquatic settings after being introduced to the environment. Propazine is in the same triazine family as atrazine, but does not adhere to soil particles as easily as other triazine compounds, giving it only moderate mobility and persistence in soil.[26] A more unique quality of propazine is its resistance to breakdown via hydrolysis and bio-degradation, while still washing into waterways to cause contamination. This is but one example of an herbicide that the statistical methods offered in this paper could also be applied to, assuming all sampling designs include outlines and patterns that contain similar parameters to each other, so that F_{ref} values may be applied correctly to both.

Polychlorinated biphenyls (PCBs) are a class of molecule that was used throughout the 1900s as a coolant in electrical transformers and other industrial processes and products. PCBs were banned in the U.S. in 1973, but their impact remains as waterways throughout the country address lingering environmental contamination. The Hudson River in New York, specifically, has battled PCB contamination for years, even investigating the impact it’s had on local amphibians.[27] Temporal adjustment factors could be applied to future Hudson River PCB studies, allowing analyses to have a standard model of sampling from which to draw conclusions. With heavy, chlorinated PCBs sinking to the bottom of the Hudson River and settling in the sediment, perhaps in shallow still-water areas where amphibians exist, future studies of PCBs and their specific effects on amphibians could require a temporal component if researchers wish to use the data quantitatively.

Environmental and aquatic contaminants that are widespread throughout the U.S. include Polycyclic aromatic hydrocarbons (PAHs). They are common in urban waterways, as exhaust from motor vehicles, gas- and oil-fired heating products, and steel mills are all significant sources of PAH contaminants.[28] PAHs have previously demonstrated toxicity to amphibians, specifically on embryos and larvae, making them an interesting target of further study. Future studies that wish to measure PAHs in the environment could employ temporal adjustment factors to cut down on discrepancies between yearly PAH concentration measurements in natural waterways, allowing more focus to be put on how the amphibians react to environmental PAH doses rather than focusing on daily sampling techniques.

Conclusion

The EPA suggests that appropriate and well-designed water monitoring studies should take into account both temporal and spatial patterns of exposure, focusing measurements on when and where an herbicide is used.[23] A daily sampling strategy followed by temporal adjustment factors and larger time-segments would serve as an approach to adjust for any temporal discrepancies in monitoring data. Carefully designed studies are necessary for any scientifically relevant information to be found from data, and aquatic atrazine analyses have not yet been standardized. Temporal adjustment factors are a step in the right direction when it comes to creating a standard to which future atrazine studies may be held.

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