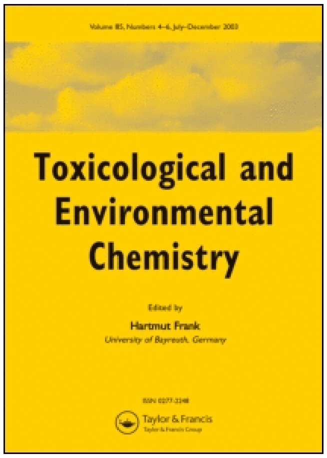

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Atrazine exposure and breast cancer incidence: An ecologic study of Missouri counties

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Atrazine exposure and breast cancer incidence: An ecologic study of Missouri counties

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Abstract
Numerous studies have implicated the triazine herbicide atrazine (6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine) as an endocrine disrupting chemical (EDC). An ecologic study of breast cancer incidence and atrazine exposure in Missouri counties was conducted for the years 1996–2002. The objective of this study was to determine if breast cancer incidence rates were associated with atrazine exposure in Missouri counties. Atrazine detections in public drinking water sources and a surrogate measure of combined acres of corn and sorghum planted were used to determine which of Missouri's 115 counties could be classified as having high *versus* low atrazine exposure. Overall, 19 counties were classified as high and 25 as low in exposure to atrazine. Linear regression analyses were conducted using covariates of median income, % black race, and incidence of high parity. Models were generated for all stages of breast cancer (localized and invasive) and for invasive cancer alone. Models refinement was conducted by stepwise elimination of the least significant predictor variables. In the final model, the high-parity predictor was statistically significant in the model for all stages of breast cancer, but not for invasive cancer.The atrazine indicator was not statistically significant in either regression equation.

Keywords: *Atrazine, breast cancer, endocrine disruption*

Introduction

The triazine herbicide atrazine is extensively used with approximately 34.5 million kg applied to cropland annually in the United States [1]. Though the International Agency for Research on Cancer categorizes atrazine in Class 3 – Not Classifiable as to Carcinogenicity in Humans [2], some epidemiological studies linked atrazine to human cancers.

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An ecologic study in England used linear regression techniques to examine atrazine application and breast cancer incidence. In the study, a positive association was found between the two in certain rural areas [3]. Another ecologic study of Kentucky counties demonstrated an increase in breast cancer risk with higher triazine herbicide exposure [4]. Breast cancer is primarily a disease occurring in women with the lifetime risk for women being 13% compared to 0.11% for men [5]. Risk for breast cancer depends on lifetime exposure to estrogens and genetic and environmental factors [6]. The established factors which increase risk for breast cancers are those related to lifetime exposure to estrogens such as (1) early age at menarche, (2) late age at menopause, (3) older age at first full-term pregnancy, (4) decreased parity, and (5) increasing age [3,7–9]. Incidence rates were also shown to differ by race, with white women having a quantitative higher incidence [10]. Higher socioeconomic status is also associated with a quantitative increase in breast cancer risk, although this may be related to differences in class reproductive factors such as number of children a woman bears. Breast cancers are classified as invasive or *in situ* (localized) with invasive cancer being the more serious of the two. In the United States, an estimated 212,920 new cases of female breast cancer were diagnosed in 2006 with 4570 in Missouri [11].

Detection of chemicals in water sources near their use are common, especially in soil types that allow for herbicide run-off [12,13]. Therefore, water sources are a potential source of human non-occupational exposure to atrazine. The US Environmental Protection Agency has set a maximum contaminant level (MCL) for atrazine at $3\,\mu g\,L^{-1}$. The combination of crop growth patterns and soil types in Missouri contributes to the vast majority of atrazine detections in treated drinking water being found primarily in the northern section of the state. Missouri ranks as a major corn and sorghum producer [14,15]. The Missouri pattern of atrazine detections in public drinking water sources make this State a likely candidate to conduct an ecologic study of breast cancer incidence and herbicide exposure at the county level. The objective of this study was to determine if breast cancer incidence rates are associated with atrazine exposure in Missouri counties.

Materials and methods

Study design and population

An ecologic study was conducted using secondary data to (1) determine measures of environmental exposure to atrazine for Missouri counties from 1996 to 2002 and (2) examine the association between these measures and breast cancer incidence using county as the sampling unit. Missouri's total population of over 5,590,000 million people is divided into 115 counties. The population consists of 51.4% female residents [16]. Migration was examined by using 2000 Census data for the number of residents who were age 5 years and older who had resided in a given county for at least 5 years.

Exposure measurements

The main source of atrazine exposure was considered to be through drinking water for this study. The Missouri Department of Natural Resources began monitoring atrazine in treated drinking water in 1995 (detection limit of $0.5\,\mu g\,L^{-1}$). The number of water tests of public water supplies for each county was highly variable, ranging from 4 to 359 tests ($n = 113$). Water suppliers were not static during the study period with closures, openings, and consolidations of suppliers. Because of changes in populations served from public water sources and the lack of data from private water sources, an additional surrogate measure of combined acreages of corn and sorghum planted was used to estimate county exposure. The detection data from 1996 to 2002 was used for analysis [17]. Combined acres of corn and sorghum were used as a surrogate measure of atrazine exposure. Crop data were obtained from the National Agricultural Statistics Service which keeps records on the estimated number of acres planted in each county [18]. Crop growth patterns and atrazine detections in treated drinking water were used to determine which of Missouri's 115 counties were the most highly exposed to atrazine and which counties had the least exposure.

Outcome measurement

Combined breast cancer incidence rates for 1996–2002 for each county were obtained from the Missouri Department of Health and Senior Services (Mo DHSS), which directs the Missouri Cancer Registry [19]. The incidence rates are reported as age-adjusted to the 2000 standard population for all stages of breast cancer and invasive breast cancer alone [20]. The cases were newly diagnosed during the study period and were from residents who identified a Missouri county as their primary residence.

Demographic covariates

Demographic covariates used in the analysis came from the 2000 Census and Missouri Department of Health and Senior Services. Demographic covariates included median income as an indicator of socioeconomic status and % black race to account for differing racial compositions of the counties. High parity in each county was accounted for by using the incidence of women (per 100) giving birth who already had 4 or more previous live births for the combined years 1996–2002 [21]. Breast cancer incidence rates were already age-adjusted.

Statistics

Linear regression analyses were conducted using breast cancer incidence rates as a response variable and atrazine exposure level and demographic variables as predictors. Models were generated using two response variables: one representing a rate of all stages of breast cancer (localized and invasive), and one for invasive cancer alone. Stepwise regression was used to select significant explanatory demographic variables while keeping the atrazine exposure variables in the models. Regression analyses were conducted with Systat 11 Statistical Software and Minitab version 14.

Results

To account for the latency period of breast cancer, the crop data from 1990 was used in the study. Growing patterns have changed little since then, and the total number of acres planted in 2002 was correlated with crop data from 1990 ($r = 0.960$). Table I summarizes the criteria for county rankings. Crop acreages planted and atrazine detection in public drinking water data for all counties were first analyzed to find median values. In order for a county to be classified as "high" in atrazine exposure, it had to exceed both the median combined crop acres planted (15,000 acres) and the median % water samples detecting positive for atrazine (18%). The counties classified as "low" had fewer than 1500 combined crop acres (the first quartile) and no atrazine detections in drinking water samples.

Table I. Atrazine exposure category. Ranges are given followed by means in parentheses. Counties ranked "high" if they exceeded both the median values of crop acreage planted (15,000 acres) for all 115 counties and the median percentage of positive detections of water (18%) in those counties with at least 1 detection. Counties were ranked "low" if the combined crop acreage was below 1400 acres (the first quartile) and atrazine was not detected in any treated drinking water samples.

Atrazine exposure category	Total number of water tests	Average of positive detections ($\mu g\,L^{-1}$)	% Positive detections	Combined corn and sorghum acres planted 1990
High ($n=19$)	56–359 (130)	0.74–1.72 (1.30)	19–74% (32%)	16,900–111,700 (36,174)
Low ($n=25$)	14–212 (65)	0	0	<1400
All counties ($n=115$)	4–359[a] (84.8)	0.59–2.59[b] (1.38)	0–42% (7.6%) {18%}[d]	0–130,000 (22,959)[c] {15,000}[d]

Notes: [a]$n=113$, Two counties missing data for water analysis.
[b]$n=42$ Counties with at least 1 positive detection.
[c]Counties with little crop acreage planted and for which the estimated acreage grown was not available were assigned 0 acres planted for the purpose of analysis.
[d]Median value.

Counties that could not be clearly differentiated into either the high or low category, such as those that had crops planted but few if any atrazine detections, were not included in the regression analysis. Using the criteria of both crop acreage and atrazine detections in drinking water to determine the counties with the highest and lowest atrazine exposure allowed for comparison of breast cancer rates between the two groups. Because previous ecologic studies found associations between atrazine and breast cancer, examining only the counties with high and low exposure would be expected to reveal an association if one is present. It should be noted that the mean concentration for all of the highly exposed counties ($1.3\ \mu g\,L^{-1}$) was well below the MCL of $3\ \mu g\,L^{-1}$. Figure 1 illustrates the counties identified as high and low-atrazine exposure in this study.

A summary of the demographic covariates is given in Table II. County migration was considered by examining % people age 5 years and older who had resided in the county from 1995 to 2000. Pulaski county (low exposure group) had the lowest % 5 year residents (52%) and the highest % black population (12%); however, fitting the model with and without this county did not change the overall results of the analyses. Examination of the data suggested natural logarithm transformations of the cancer incidence rates and the high-parity incidence rates to correct skewed, linear relationships among the two variables, and to normalize residuals. Counties were coded high (1) or low (0) in atrazine exposure. Initial box plots of the transformed breast cancer rates in the high and low categories showed much overlap in the data sets (Figure 2). The model was fit using all covariates and interaction terms between the atrazine indicator and each term. Stepwise regression procedures selected only the transformed high-parity incidence as a significant demographic covariate. As stated previously, the atrazine exposure was kept in the model.

The final model for the regression for all stages of breast cancer is given by:

$$\ln(bc) = 5.02 - 0.122\ \ln(\text{births}) + 0.0526\ \text{atz indicator}$$

The model for invasive breast cancer is given by:

$$\ln(\text{invasive}) = 4.84 - 0.0969\ \ln(\text{births}) + 0.0332\ \text{atz indicator}$$

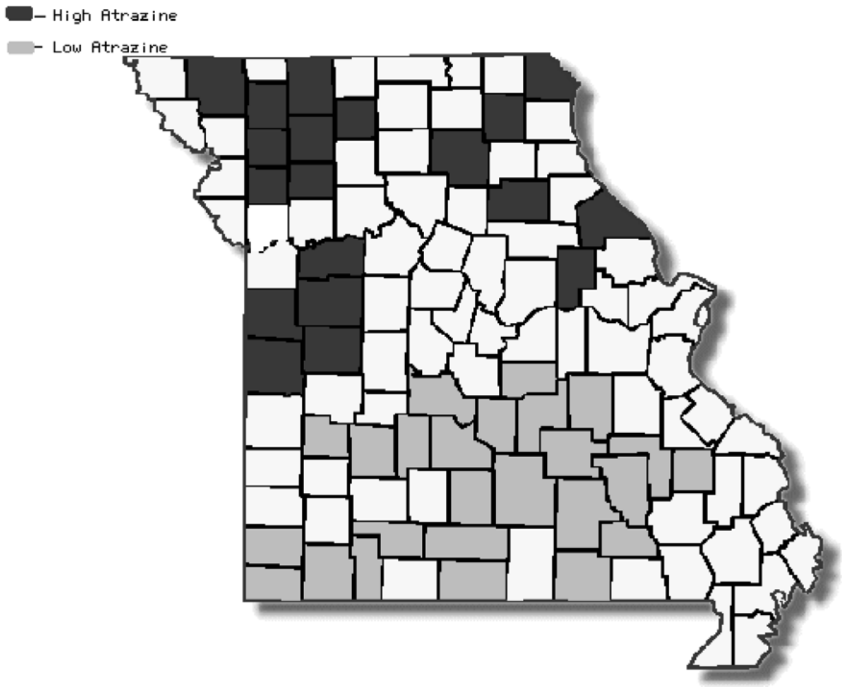

Figure 1. Map of Missouri counties coded high and low in atrazine exposure. High category: Bates, Caldwell, Cass, Clark, Clinton, Daviess, DeKalb, Gentry, Grundy, Harrison, Henry, Johnson, Knox, Lafayette, Macon, Monroe, Montgomery, Nodaway, and Pike. Low category: Barry, Camden, Carter, Cedar, Christian, Crawford, Dallas, Dent, Douglas, Iron, Laclede, McDonald, Madison, Maries, Newton, Oregon, Ozark, Phelps, Polk, Pulaski, Reynolds, Shannon, Stone, Texas, and Wright.

Where: bc = breast cancer incidence (all stages)

invasive = invasive breast cancer

births = high − parity predictor (rate of women having had $\geq$ 4 previous live births)

atz indicator = atrazine indicator, coded 0 if low and 1 if high

The high-parity predictor was statistically significant in the model for all stages of breast cancer ($p = 0.018$), but not in the model for invasive cancer ($p = 0.077$). The atrazine indicator was not statistically significant in either regression equation ($p = 0.167$ for all stages and $p = 0.371$ for invasive breast cancer). Table III summarizes the atrazine indicator coefficient in the models. Coded scatter plots of the response variable *versus* the transformed high-parity predictor variable visually show the non-significant interaction between the two terms (Figure 3). Although the atrazine indicator variable was not significant in the model, the graphs do show a pattern of increased breast cancer incidence in the high-atrazine groups. Returning to the original scale, the incidence of all stages of breast cancer in the high-exposure counties is estimated to be 1.05 times that of the low-exposure counties, after adjusting for high parity. An approximate 95% confidence interval for the increased incidence is (0.98, 1.14) which covers 1. Similar results for invasive breast cancer incidence are an estimate of 1.03 times with a 95% confidence interval of (0.96, 1.11), also covering 1.

Table II. Demographic information for exposure categories of atrazine: Data ranges are given followed by means in parentheses.

Atrazine exposure category	Total population	Breast cancer incidence/100,000 women	Invasive breast cancer/100,000 women	$\geq$4 Births/ 100 women	% Black population	Median income	% In county 5 years
High ($n=19$)	364,913	103.5–164.5 (132.0)	90.5–143.4 (113.9)	2.8–11.7 (4.93)	0.10–8.6 (0.02)	25,601–49,562 (32,369)	61–85 (76)
Low ($n=25$)	562,223	104.4–160.6 (129.7)	91.9–133.5 (112.4)	1.8–8.4 (3.94)	0–12 (0.008)	20,878–38,085 (28,736)	52–81 (74)

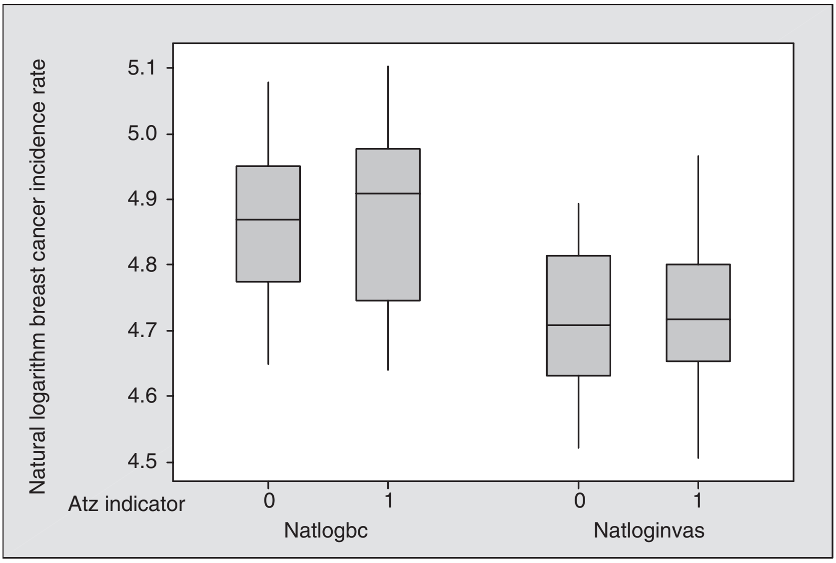

Figure 2. Boxplots of transformed breast cancer incidence rates for low (0) and high (1) atrazine exposure.

Table III. Analysis of atrazine indicator (β_2) in model.

Model	Coefficient of atrazine indicator (ß$_2$)	p-value	Standard Error of Coefficient	e^{β_2}	Degrees of freedom	95% CI for (β_2)	95% CI for e^{β_2}	R^2 for model
Breast cancer	0.0523	0.167	0.0374	1.055	40	1.137, −0.0230	3.117–0.977	14.1
Invasive breast cancer	0.0332	0.371	0.0367	1.034	39	0.1074, −0.0410	1.113–0.960	8.3

Notes: Confidence Intervals (CI) based on t-distribution. The inverse of natural logarithm of ß$_2$ gave 95% CI for both regression equations which includes 1, supporting the conclusion that the differences in cancer rates between the high atrazine and low atrazine exposure groups are not significant.

A second confirmatory analysis was conducted matching high- and low-exposure counties on high-parity incidence and conducting a non-parametric Wilcoxon signed rank test on the matched pairs. The overall conclusions for the analysis were the same.

Discussion

The objective of this ecologic study was to determine if county atrazine exposure was statistically significant in either model of breast cancer incidence. The difference in breast cancer rates between counties classified as high in atrazine exposure and those classified as low in exposure was not statistically significant. The ecologic study design is limited in its use of aggregate data rather than individual level data. An additional challenge here was assigning an exposure category to the counties. The technique used here selected counties with exposures that were distinguishable in the data, but this required dropping some

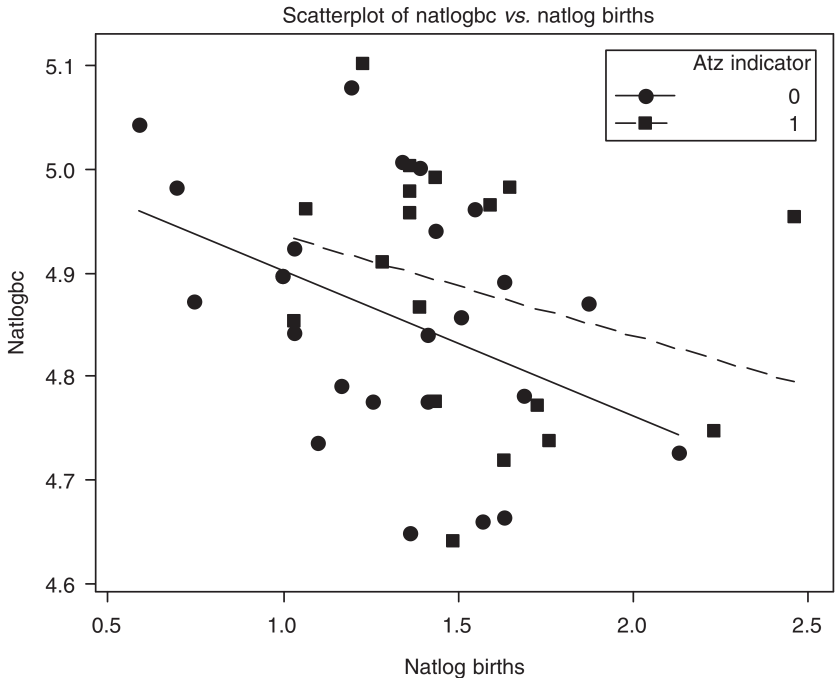

Figure 3. Scatterplot of natural logarithm of breast cancer incidence *vs*. natural logarithm of high-parity predictor variable. No interactions are apparent between the predictor and response variable. Although not statistically significant, the general trend is for the high-atrazine counties (atz indicator = 1) to have higher breast cancer incidence than the low-atrazine counties.

counties from the analysis that could have provided additional insight if their exposure levels were known. Ecologic studies may, however, be used to make inferences about group rates [22]. Although not able to establish causality, they are useful for initial examination of a health outcome and environmental factor [23]. Another factor that influences studies such as this is the difficulty in determining what other factors influence the outcome measure. The question of environmental exposure to pesticides is difficult to assess, because of the plethora of confounding factors including multiple sources of exposure and possible interactions with other environmental agents. The effects of confounding, due to unobserved latent variables, are large and introduce biases that favor or detract from an apparent association in observed data [24]. Therefore, a lack of association between breast cancer incidence and atrazine exposure found in an ecologic study cannot rule out the possibility that such an association exits, just as an observed association could not be considered confirmatory in a single study. Associations in observational studies are generally confirmed only after they appear consistently in different studies, in differing contexts, and in analogous lab experiments where assignments of treatments to experimental units can be randomized [25].

The results of this study are consistent with a number of other studies in other states in this country that did not detect a significant association between atrazine and breast cancer risk. Women occupationally exposed to atrazine would be expected to have a higher exposure to atrazine than the general population. A California study of Hispanic females, who compose a high % agricultural work force, found no association between atrazine and breast cancer [9]. In a prospective cohort study of farmer's wives, breast cancer risk was not increased in either women who had applied triazines or whose husbands had applied triazines [26]. An ecologic study conducted in Kentucky expanding on the work of Kettles et al. [4] found no association between breast cancer and atrazine at the county level [27]. In the study by Muir et al. [3] which used linear regression techniques to compare breast cancer incidence and atrazine applications in wards of English counties, the association between the pesticide and breast cancer incidence was not consistent for all the areas examined.

The question of environmental agents influencing health outcomes is complex. Ecologic studies assess aggregate data and thus have limitations. However, these studies, including ours, contribute to the greater understanding of the environmental impact of environmental chemicals.

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