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Risk of breast cancer for women living in rural areas from adult exposure to atrazine from well water in Wisconsin

JANE A. MCELROY[a], RONALD E. GANGNON[b], POLLY A NEWCOMB[a,c], MARTY S. KANAREK[a,d], HENRY A. ANDERSON[e], JIM VANDEN BROOK[f], AMY TRENTHAM-DIETZ[a,d] AND PATRICK L. REMINGTON[a,d]

[a]*University of Wisconsin Comprehensive Cancer Center, Madison, WI, USA*
[b]*Departments of Biostatistics and Medical Informatics, University of Wisconsin, Madison, WI, USA*
[c]*Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA*
[d]*Department of Population Health Sciences, University of Wisconsin, Madison, WI, USA*
[e]*Wisconsin Division of Public Health, Department of Health and Family Services, Madison, WI, USA*
[f]*Wisconsin Department of Agricultural, Trade and Consumer Protection, Groundwater Protection Unit, Madison, WI, USA*

Research has suggested possible human health effects from low-level widespread exposure to environmental contaminants. We employed a novel exposure estimation technique using a publicly available data set to examine atrazine exposure, a suspected endocrine disruptor, in relation to breast cancer risk for women living in rural areas of Wisconsin. Incident breast cancer cases who were 20–79 years of age from 1987 to 2000 ($n = 3{,}275$) and living in rural areas of Wisconsin at the time of interview were identified from Wisconsin's statewide cancer registry. Female controls of similar age and living in rural areas of Wisconsin were randomly selected from population lists ($n = 3{,}669$). The addresses at diagnosis or reference year of study participants were assigned latitude/longitude coordinates (geocoded). The results from three statewide random studies of atrazine levels in well water in 1994, 1996, and 2001 were obtained from the Wisconsin Department of Agriculture, Trade, and Consumer Protection. Natural neighbor interpolation was used to estimate atrazine exposure levels separately for each of the 3 years. The mean atrazine exposure level was assigned to each participant based on her geocode. After adjustment for established breast cancer risk factors, compared to women in the lowest category of atrazine exposure (<0.15 ppb), the odds ratio of breast cancer for women exposed to atrazine concentrations of 1.0–2.9 ppb was 1.1 (95% CI 0.9–1.4). Results from this large population-based study do not suggest an increased risk of breast cancer from adult exposure to atrazine in drinking water. The possible risk for women exposed to levels of atrazine at or above statutory action levels of ≥3 ppb (OR 1.3, 95% CI 0.3–6.5) could not be ruled out due to small numbers in this category.
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Introduction

Public concern over contaminated drinking water from pesticides, in particular, the widely used corn herbicide atrazine, has led the US Environmental Protection Agency's (USEPA) Office of Water to regulate the compound under the Safe Drinking Water Act in 1991. Water ingestion is thought to be the major route of atrazine exposure for people who have had no occupational contact (Cooter and Hutzell, 2002). No biomarker which would signal integrated lifetime exposure to atrazine has been identified, and atrazine is not known to bioaccumulate in the body. Concentrations of residues in urine peak approximately 12 h after exposure and decreases rapidly, nearly to baseline after 24 h (Catenacci et al., 1993). Therefore, monitoring levels of the pesticide in the environment, specifically in water samples, represents a potential mechanism to assess human exposure.

Toxicological studies using amphibians and rodents have reported endocrine disrupting effects (Hayes et al., 2002) and suppression of the immune system (Yang et al., 1989; Porter et al., 1999) from water contaminated with atrazine at levels found in drinking water. Recent rodent studies have suggested atrazine exposure has the potential for enhancing the growth of mammary tumors, partly through increasing cell proliferation in the promotion/progression stage (Ueda et al., 2005) and modulation of cell-mediated immune function (Karrow et al., 2005). However, after extensive hearings and study of the research findings, the Cancer Assessment Review Committee sponsored by the USEPA recently classified atrazine as not likely to be a carcinogen in humans (Baetcke et al., 2000). In contrast, the International Agency for Research on Cancer has concluded that atrazine is not classifiable as to its carcinogenicity in humans due to insufficient evidence (IARC, 1999). Contradictory results have been reported from population based epidemiologic

1. Address all correspondence to: Dr. Jane A. McElroy, University of Wisconsin Comprehensive Cancer Center, Room 307 WARF, 610 Walnut St, Madison, WI 53726, USA. Tel: +1 608 265 8780.
Fax: +1 608 265 5330. E-mail: jamcelroy@wisc.edu
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studies that evaluated risk of breast cancer associated with atrazine exposure from drinking water contamination (Kettles et al., 1997; Hopenhayn-Rich et al., 2002; Muir et al., 2004).

To evaluate the relation between atrazine and breast cancer, we used data from three sequential agricultural chemical monitoring studies completed in 1994, 1996, and 2001. These studies randomly sampled water from private wells located in rural areas of Wisconsin for atrazine contamination levels (LeMasters et al., 1995; LeMasters and Baldock, 1997; Vanden Brook et al., 2002). We used natural neighbor interpolation technique to assign atrazine exposure values to study participants who had participated in one of three sequential breast cancer case–control studies initiated in 1988, 1992, and 1997 and lived in rural areas of Wisconsin.

Methods

Study Participants

All study participants ($n = 22{,}495$) were female, English-speaking Wisconsin residents at the time of the study, 20–79 years of age with a listed telephone number. All women received a letter of introduction from the study center's principal investigator, followed by phone contact. Human subjects' approval was received for this project from the University of Wisconsin-Madison Institutional Review Board. The study methods are described in more detail in previous reports (Newcomb et al., 1994; Newcomb et al., 1999; McElroy et al., 2004). Briefly, the three sequential studies' primary goals were to evaluated modifiable lifestyle risk factors: alcohol consumption and breast feeding habits (1988–1991); hormone replacement therapy use (1992–1995); and finally, physical activity patterns (1997–2001).

Incident invasive breast cancer cases were identified from Wisconsin's mandatory statewide tumor registry from 1988 to 2001. Of 13,213 potential case women, 83% participated in the study (3.8% were excluded at their physician's request, 4.7% had died before contact, 1.1% could not be located, and 7.1% declined to participate). Of the 11,008 interviews conducted with case participants, seven were considered unreliable by the interviewers and were not included in these analyses.

Controls were selected randomly to yield an age distribution similar to that of the cases from lists obtained from the Wisconsin Department of Transportation (20–64 years of age) and the US Health Care Financing Administration (65–79 years of age). Control eligibility was further limited to subjects who had a listed telephone number and did not report a previous diagnosis of breast cancer. Of 13,841 potential control women, 83.1% participated in the study (1.4% had died, 1.8% could not be located, and 13.7% declined to participate). Of the 11,502 interviews conducted with control women, eight were considered unreliable by the interviewers and were not included in these analyses.

Participants' mailing addresses at the time of the interview were assigned latitude/longitude coordinates to the address point location (97%) with an 80 percent spelling and overall sensitivity score, the nine digit line segment centroid (1%), or the zip code centroid (2%). See McElroy et al. (2003) for more details on the geocoding methods (McElroy et al., 2003).

Structured 40-min telephone interviews, conducted between September 1988 and May 2001, elicited information on known or suspected risk factors for breast cancer (the instruments were comparable in all three studies), including exogenous hormone use, reproductive experiences, physical activity, alcohol consumption patterns, medical and family history, and demographics prior to an assigned reference year — approximately 1 year before diagnosis or reference. For cases, this reference year was the date of diagnosis of the breast cancer. For comparability, control subjects were assigned a reference date based on the dates of diagnosis corresponding to similarly aged cases (within 5-year strata). The reference age was defined as the subject's age at the time of the reference date. Trained study staff conducted interviews by telephone without prior knowledge of the subjects' disease status. The interviewers reported they were unaware of the case–control status for 88.0% of the cases and 79.1% of the controls until the end of the interview.

Subjects for Analysis

Analysis was limited to women who lived in rural areas of Wisconsin, defined as their residential city, village, or town that did not have a public water system (3,275 cases and 3,669 controls).

Agricultural Chemical Data Set

The Wisconsin Department of Agriculture, Trade and Consumer Protection (WDATCP) conducted the Atrazine Rule Evaluation Studies in 1994, 1996, and 2001 with 289, 278, and 336 wells sampled, respectively. See LeMasters et al. (1995), LeMasters and Baldock (1997), Vanden Brook et al. (2002) for more details on these studies. The purpose of these representative statewide surveys of a random sampling of wells was to provide information on the extent and distribution of agricultural chemicals, such as atrazine, in groundwater throughout the state (Baldock, 1993). Wells were selected using a stratified random sampling procedure. Nine agricultural statistical districts, which are groups of adjoining counties, formed the sampling strata. The number of samples collected in each district was based on the number of farms in the district and atrazine concentration variability from previously sampled wells which ranged from as few as 18 unique wells in one agricultural district (SouthEast) to as many as 118 unique wells in another agricultural district (SouthCentral) over the three study periods. To allocate the samples in each district, a random sample was drawn from a list of all the civil sections (excluding those covered by water or publicly owned). Civil sections were used because no comprehensive current list of private wells exists. In each civil section, a random 10-acre parcel was selected and the well nearest its center was identified to represent the groundwater of the civil section. The parcel was visited to determine if a private well existed within those 10-acres. If so, three attempts were made to contact the owner and determine their willingness to participate in the survey. If no acceptable well (e.g., no water treatment used) or willing owner was found within the entire section, a replacement section and random 10-acre plot was selected. The contact protocol was repeated until a well water sample was obtained. Virtually all the samples were collected in the months of May to October for the 1994 and 1996 studies and from October to April in the 2001 study.

For surveys conducted in 1996 and 2001, a 50% sample rotation scheme in which half of the wells in the previous study were selected and 50% were new wells equally distributed geographically. The water samples from private wells were obtained only when no water treatment system was used; water treatment for private well water is uncommon (personal communication, Jim VandenBrook, section chief Department of Agriculture, Trade, and Consumer Protection 12/09/2005). Water was collected through the cold water faucet after letting the water run for 10 min. This sample was analyzed using gas chromatography for the parent compound and its metabolites: deethylatrazine, deisoatrazine, and diaminoatrazine metabolite by the WDATCP Bureau of Laboratory Science. A combination of these metabolites and parent compound provided the total chlorinated residues of atrazine value.

Well addresses were recorded using the Public Land Survey System, a nationwide survey in which the land in each state is divided into a 36-square mile parcel. These township parcels are subdivided into 40 acre plots, known as quarter-quarter sections measuring one-quarter by one-quarter mile. For each well, the centroid of this 40-acre plot was the recorded location.

Estimation Approach

Natural neighbor interpolation (Sibson, 1981) was used to estimate atrazine exposure levels across the entire state, separately for each year. Atrazine exposure is defined as an exposure to an interpolated concentration of atrazine residues in residential drinking water. Natural neighbor interpolation uses a weighted moving average of surrounding or neighboring data points to compute the interpolated values. This method creates a surface of estimated atrazine exposure values. Neighboring points and the corresponding weights are based on the Voronoi diagram of the data points. The interpolation was performed using ArcGIS 9.0 spatial analysis tool (Environmental Systems Research Inc., Redwood, CA, USA). An averaged atrazine exposure level — over the time periods (1994, 1996 and 2001) — was assigned to each participant.

Analysis

We used odds ratios and 95% confidence intervals from logistic regression models to estimate relative risks of breast cancer incidence (Breslow and Day, 1980). We analyzed atrazine exposure as a categorical variable (<0.15, 0.15–0.5, 0.51–1.0, 1.01–2.99, ≥ 3.0 ppb) defined a priori based on potential clinical and policy relevance and as a continuous variable. For regression models, the reference category was defined as subjects with the lowest level of atrazine exposure.

Covariates in the models included established risk factors for breast cancer. Age was defined as age at diagnosis for cases and age at a similar reference date for controls (continuous). Family history of breast cancer was defined as a reported diagnosis in a mother, sister, or daughter (absent, present, unknown). Recent alcohol consumption was defined as the total number of drinks of beer, wine, and hard liquor consumed per week 3 or 5 years before the reference date (three categories). Parity was number of full-term pregnancies (defined as pregnancies of >6 months' gestation resulting in a live birth or stillbirth) (three categories). Postmenopausal status was assigned to women reporting having undergone natural menopause or a bilateral oophorectomy before the reference date. Women were also categorized as postmenopausal if they had a hysterectomy alone and were 55 years or older (equal to the 90th percentile of age at natural menopause among the controls). In cases of hysterectomy without bilateral oophorectomy, menopausal status was considered unknown if the woman's reference age was <55 years (three categories — premenopausal; postmenopausal; unknown). Age at first full-term pregnancy (five categories), body weight index defined as self-reported weight (kg) divided by height (m) squared (five categories), age at menopause (five categories), study wave (three categories), and education (five categories) were also covariates in the model. Models were developed for participants living in rural areas throughout Wisconsin and for those participants living in the south central agricultural region of the state which comprises the contiguous counties of Columbia, Dane, Dodge, Green, Jefferson, and Rock — the agricultural district in Wisconsin with the highest atrazine application amounts.

Temporal agreement of atrazine levels was evaluated using Pearson's correlation coefficients for those wells in which samples were taken in subsequent years — 1994 atrazine values with the 1996 atrazine values ($n=140$ wells resampled) and the 1996 atrazine values with the 2000 atrazine values ($n=119$ wells resampled).

Results

Compared with controls, women with breast cancer were more likely to have established risk factors for breast cancer including a later age of first birth, fewer children, a family history of breast cancer, a higher body mass index during postmenopausal years, a later age of menopause, higher rates of alcohol consumption, and more education (Table 1).

For the sampling years of 1994, 1996, and 2001, 20%, 16%, and 14%, respectively, of the wells sampled had detectable atrazine with approximately half located in the south central agricultural district. Of the 289, 278, and 336 wells sampled, 3%, 3%, and 1% of the wells, respectively, had atrazine values of over 3 ppb (Table 2). The statewide atrazine concentration levels significantly declined from 1994 to 2001 (Vanden Brook et al., 2002). The highest agricultural region in Wisconsin for atrazine detects was South Central with 34% of the wells at $\geq$0.15 ppb and the mean atrazine concentration in well water was also the highest at 0.48 ppb (Table 2).

The odds ratio of breast cancer for women who were exposed to atrazine concentrations of 1.0–2.9 ppb as compared to women in the lowest exposure category (<0.15 ppb) was 1.1 (95% CI 0.9–1.5) — age adjusted only model. This odds ratio did not change after adjustment of known and suspected breast cancer risk factors (Table 3). Few women had exposure to atrazine at $\geq$3.0 ppb with the odds ratio in the fully adjusted model of 1.3 (95% CI 0.3–5.0) (Table 3). Similar odds ratios were observed for rural women living in high atrazine use area of the South Central Agricultural District (Table 3). No significant interactions were discerned between risk factors (Table 1) and atrazine exposure (data not shown).

Pearson's correlation showed strong relations between atrazine concentrations in wells sampled in 1994 and then again in 1996 ($r = 0.79$; 95% CI 0.72–0.84) and those sampled in 1996 and then again in 2000 ($r = 0.72$; 95% CI 0.62–0.80). Mean atrazine levels decreased from 1994 to 1996 (−0.09 ppb) and from 1996 to 2000 (−0.01 ppb).

Discussion

The results from this large case–control study do not suggest an increased breast cancer risk from exposure to low levels of atrazine found in wells sampled in Wisconsin. We lacked sufficient power to determine whether an association existed for breast cancer risk for women at the highest category of 3.0 ppb or more. Our confidence in these results is strengthened by the large sample size, high response rates, careful modeling of atrazine exposure in rural areas, and information on individual-level risk factors.

Two epidemiologic studies have specifically evaluated atrazine exposure from drinking water and breast cancer risk. These population-based studies were limited to exposure estimation based on large geographic areas rather than specific drinking water wells. Kettles et al. (1997) reported a statistically significant increase in breast cancer risk (OR = 1.14 and 1.20, respectively) for women living in

Table 1. Characteristics of rural women in Wisconsin with breast cancer and controls aged 20–79

Risk factors	Cases ($n = 3,275$)		Controls ($n = 3,669$)		Odds ratio[a]	95% CI
	Number	%	Number	%		
Age at birth of first child (year)						
<20	615	19	758	21	1.0	
20–24	1,422	43	1,698	46	1.0	0.9–1.2
25–29	629	19	621	17	1.2	1.1–1.5
30+	223	7	226	6	1.2	1.0–1.5
Nulliparious	386	12	366	10	1.3	1.1–1.6
Parity						
0–1	671	20	629	17	1.0	
2	819	25	855	23	0.9	0.8–1.1
3	732	22	829	23	0.8	0.7–0.9
4+	1,030	31	1,336	36	0.7	0.6–0.8
Family history of breast cancer						
Absent	2,538	77	3,118	85	1.0	
Present	647	20	457	12	1.7	1.5–2.0
Unknown	90	3	94	3%	1.2	0.9–1.6
Body mass index (kg/m²)[b]						
1 (<21.4)	265	8	353	10	1.0	
2 (21.5–23.5)	355	11	414	11	1.1	0.9–1.4
3 (23.6–26.5)	566	17	610	17	1.2	1.0–1.5
4 (>26.6)	956	29	1,012	28	1.2	1.0–1.5
Unknown	78	2	90	2	1.1	0.8–1.5
Menopausal status						
Premenopausal	879	27	964	26	1.4	1.2–1.6
Postmenopausal	2,220	68	2,479	68	1.0	
Unknown	176	5	226	6	1.1	0.9–1.3
Age at menopause (year)[b]						
<45	427	13	558	15	1.0	
45–49	493	15	534	15	1.2	1.0–1.4
50–54	730	22	828	23	1.1	0.9–1.3
55	276	8	256	7	1.3	1.1–1.6
Unknown	294	9	303	8	1.2	1.0–1.5
Alcohol consumption per week						
0 drinks	582	18	711	19	1.0	
Up to 7.0 drinks	1,014	31	1,259	34	1.0	0.9–1.2
More than 7.0 drinks	1,645	50	1,653	45	1.3	1.1–1.4
Unknown	34	1	46	1	0.9	0.6–1.4
Education						
<12th grade	479	15	529	14	1.0	
High school graduate	1,502	46	1,747	48	1.0	0.9–1.2
At least some college	1,265	39	1,356	37	1.2	1.0–1.3

[a]Age adjusted only.
[b]Postmenopausal women only.

Table 2. Characteristics of well water sampling of atrazine in Wisconsin

	Years sampled					
	1994		1996		2001	
	Number of wells ($n=289$)	%	Number of wells ($n=278$)	%	Number of wells ($n=336$)	%
Concentration (in ppb)						
<0.15	230	80	233	84	288	86
0.15–0.50	9	3	10	4	10	3
0.51–1.0	21	7	10	4	17	5
1.01–2.99	19	7	18	7	18	5
≥3.0	10	3	7	3	3	1
Mean	0.36		0.27		0.18	
Median	0.00		0.00		0.00	
Maximum	13.63		12.47		6.26	

	Atrazine concentration categories averaged over 3 time periods						
	<0.15 (%)	0.15–0.50 (%)	0.51–1.0 (%)	1.01–2.99 (%)	≥3.0 (%)	Mean (in ppb)	Max (in ppb)
Agricultural							
Central	77	5	8	7	3	0.43	8.05
East Central	92	3	3	3	0	0.06	1.08
North Central	100	0	0	0	0	0.00	0.00
North East	97	3	0	0	0	0.02	0.46
North West	98	0	2	0	0	0.02	0.88
South Central	66	7	11	13	3	0.48	5.50
South East	90	0	5	5	0	0.08	1.20
South West	85	4	3	5	2	0.20	4.15
West Central	86	4	4	4	2	0.26	13.63

Table 3. Multivariate odds ratio for breast cancer for rural women living in Wisconsin according to atrazine exposure

Atrazine exposure (in ppb)[a]	Cases number	%	Controls number	%	Odds ratio[b]	95% CI[b]	Odds ratio[c]	95% CI[c]
State of Wisconsin	($n=3,275$)		($n=3,669$)					
<0.15	2,570	78.5	2,890	78.8	1.0		1.0	
0.15–0.5	401	12.2	447	12.2	1.0	0.9–1.2	1.0	0.9–1.2
0.51–1.0	159	4.9	189	5.2	0.9	0.8–1.2	1.0	0.8–1.2
1.01–2.99	140	4.3	139	3.8	1.1	0.9–1.5	1.1	0.9–1.4
≥3.0	5	0.2	4	0.1	1.4	0.4–5.1	1.3	0.3–5.0
High atrazine use area[d]	($n=407$)		($n=436$)					
<0.15	121	29.7	128	29.4	1.0		1.0	
0.15–0.5	143	35.1	142	32.6	1.1	0.8–1.5	1.1	0.7–1.5
0.51–1.0	69	17.0	83	19.0	0.9	0.6–1.3	0.9	0.6–1.3
1.01–2.99	70	17.2	80	18.3	0.9	0.6–1.4	0.9	0.6–1.4
≥3.0	4	1.0	3	0.7	1.3	0.3–5.9	1.2	0.3–5.8

[a]Categories are based on potential clinical and policy revelance.
[b]Adjusted for age only.
[c]Adjusted for age, parity, phase of study, case-control status, age of menopause, body mass index, age at first full term pregnancy, menopausal status, age at menarche, recent alcohol consumption, education.
[d]South Central Agricultural Statistics District: Columbia, Dodge, Dane, Jefferson, Green, and Rock counties.

Kentucky counties designated as medium or high levels of atrazine exposure based on a county exposure summary index and breast cancer incidence rates (1993–1994). This index was composed of water contamination data — non-random groundwater collection data from both private and public water systems and surface water collection for public water systems, — acres of corn production, and pesticide use data from a survey of pesticide appliers.

In contrast to this suggestive finding, Hopenhayn-Rich et al. (2002) found no association using a similar summary index for Kentucky for breast cancer incidence averaged over a 5-year time period, 1993–1997. This summary index was comprised of water contamination data (public water systems only), atrazine sales in 1997, and acres of corn production.

Another recent ecological study in England used breast cancer incidence rates and amount of applied atrazine (in kilograms of active ingredient used) in two agricultural counties. Muir et al. (2004) found no overall spatial association but did find a statistically significant positive spatial association between atrazine use and breast cancer in the rural areas of the counties. Thus, the epidemiologic data are both limited and apparently inconsistent.

Several factors support the use of water sampling to estimate atrazine exposure. Atmospheric concentrations are very low, in the nanogram range, and air monitoring data after pesticide application and proximal to application sites suggest significant decreases in concentration levels within a mile (Gunier et al., 2001). In our study, few women ($n = 106$) lived within one mile of the wells tested. Atrazine slowly degrades once it reaches the groundwater, and the chlorinated degradation products are considered as harmful to human health as the parent product (Chesters, 1991). Atrazine has not been detected in market basket surveys as a food residue (Berry et al., 1997), except in the occasional atrazine applicator's meal at pesticide application time (Melnyk et al., 1997). Consequently, the atrazine concentration in the water can be assumed with reasonable confidence to be the concentration level delivered to consumers.

We used well water samples from randomly selected wells in three linked environmental monitoring studies over an 8-year period. Exposure levels were averaged over three testing periods and these values were assigned to participants' geographic location at the time of their diagnosis. Persistence of atrazine contamination in water over time allows for use of multiyear sampling data. Once atrazine reaches the aquifer, half-life has been reported to be >10 years (Chesters et al., 1993). Statewide atrazine application amounts have been declining from approximately five pounds applied per acre in the 1970 to approximately one pound applied per acre in the 2000s (Wisconsin Statistical Reporting Service, 1972; Wisconsin Agricultural Statistics Service, 2001). Wisconsin private wells respond relatively rapidly to surface applications or lack thereof. This is demonstrated by the rapid reduction in atrazine concentrations in the majority of wells in atrazine prohibition areas (i.e., use of atrazine is prohibited in a one-mile by one-mile area around a tested well found to have atrazine levels at or above 3.0 ppb; the centroid of the area is the location of the sampled well where atrazine was detected at or above 3 ppb.) of 5 years or less for many wells. Also, some private wells in the 2001 survey showed acetochlor metabolite detections just 6 years after the product was introduced in the state (Vanden Brook et al., 2002). So, it could be speculated that atrazine levels might have been higher in the 1970s and 1980s than detection levels reported from the 1994 survey (allowing some lag time from application to appearance in wells). Off-season atrazine detection in water is clearly linked to its persistence (degradation speed). Half-life values from laboratory experiments range from 2 weeks to several months (Wauchope, 1978; Chau and Afghan, 2000). However, in reality, atrazine's persistence in the field (field meaning outside of the laboratory) appears to be biphasic. When applied in the spring, initial rapid degradation occurs during the first 2 months after application, followed by slower degradation in the dry summer and cold fall and winter in the Midwestern states (Koskinen and Clay, 1997). Soil type, initial and seasonal water content, pH, microbial population density, temperature (Koskinen and Clay, 1997) but not concentration (Gan et al., 1994) have been shown to influence the rate of both rapid and slow degradation phases. Therefore, we believe our measurement is a reasonable time-integrated measure of atrazine exposure at the rural participants' residences.

There are some limitations that need to be considered when evaluating this study. For this analysis, the classification system may not accurately reflect a woman's actual exposure to atrazine and as described by Vineis, non-differential misclassification of exposure may depress the odds ratio (Vineis, 2004). For example only home water supply was considered as the source of atrazine exposure. However, a significant amount of water — from non-well sources — could have been consumed outside the home (Shimokura et al., 1998). Variations in tap water consumption patterns were not assessed. Further, we did not obtain tap water samples from the study participants and therefore, were unable to evaluate exposure directly.

Another source of misclassification is reflected in the mobility of the population. We suspect our population was reasonable stable as reflected in analyses of another population based breast cancer case control study of similar design ($n = 4{,}380$) conducted from 2001 to 2004. We were able to evaluate mobility of Wisconsin women through the collection of lifetime residential history. In this study, approximately three quarters of the women had maintained the same residence for the previous 10 years from interview date; 60% for the previous 15 years from interview date; and half of the participants had maintained the same address for the previous 20 years from interview date. From the opposite perspective, $<10\%$ of the population had moved to their residence within 2 years of the interview date.

Thirdly, the mechanism of action for atrazine carcinogenicity is unknown. Evaluation of the influence of atrazine exposure at potential critical periods (Rayner et al., 2004), such as during infancy, childhood, or adolescence can not be yet determined because atrazine's extensive use started in the 1970s (Wisconsin Statistical Reporting Service, 1972; Wisconsin Agricultural Statistics Service, 2001) and these three sequential study interviews ended in 2001. The participant's mean age was 56. As breast cancer is clearly associated with age, at this time only adults who may have been exposed to atrazine could be evaluated for breast cancer risk. Extrapolating from rodent models, Rayner et al. (2004) hypothesized that daughter's exposure to atrazine in the womb may affect her mammary gland development. They suggest that this atrazine exposed tissue may not reach full maturity (differentiated state) and therefore may be more susceptible to carcinogenesis. In another 20 years, the women who were exposed to atrazine throughout their lives, including *in utero* will have moved into the age category in which breast cancer diagnosis is much more common and therefore lifetime risk of atrazine exposure and breast cancer can be more thoroughly examined. For now, we were able to evaluate the risk of atrazine exposure for women exposed during their adult life.

A third limitation is the heterogeneity of the atrazine values and detection. Atrazine concentrations in groundwater vary horizontally, vertically, and with time as contamination flows with groundwater recharge water from a field of atrazine application to a well. Depending on the location of a participant's well, contamination may or may not enter the water supply. For example, two wells drilled very close to one another, but at different depths could yield very different atrazine values. One well may intersect the plume of contamination flowing in groundwater and the other may be above or below the plume and thus avoid the contamination. Despite this, it has been demonstrated that the probability of atrazine contamination in a given well increases in proximity to other wells of known contamination (Vanden Brook et al., 2002).

Finally, the targeted population for these environmental monitoring studies was described as groundwater in rural areas where private well use predominates (Vanden Brook et al., 2002). Consequently, we limited our analyses to rural women in Wisconsin (27% of our study population), who would very likely use private well water, not a community water system. These results cannot be generalized to women living in urban areas.

In conclusion, this report evaluating breast cancer and exposure to atrazine is the first epidemiologic study that was able to control for known and suspected breast cancer risk factors. Overall, exposure to atrazine, using agricultural chemical monitoring data of well water in rural areas and geocoded addresses at diagnosis or reference year, does not suggest an association between low-level atrazine exposure and breast cancer risk for rural women, overall. However, possible risk for women exposed to levels of atrazine at or above statutory action levels (≥ 3 ppb) could not be ruled out due to small numbers in this category.

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