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Regional Assessment of Atrazine Exposure and Incidence of Breast and Ovarian Cancers in Kentucky

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Abstract. The association between breast and ovarian cancers with endogenous estrogen or hormonally related events has led to the hypothesis that exposures to exogenous estrogenic compounds in the environment may increase the risk of these cancers. Atrazine, the most commonly used herbicide in the United States, belongs to this group of compounds and is widely used in corn production. This study is an expansion of a previous investigation conducted in Kentucky. Using secondary data, we derived several indices of environmental exposure to atrazine and examined the association between these measures and the incidence of breast and ovarian cancer in Kentucky over a 5-year period (1993–97). Exposure indices to atrazine were derived based on public water measurements, acres of corn planted, and pounds of atrazine sold. Data on breast and ovarian cancer incidence were obtained from the Kentucky Cancer Registry by county and by the 15 Area Development Districts (ADDs) in which the 120 counties are grouped. Poisson regression analyses adjusted for education and race were conducted separately for each index of exposure and for a combined total exposure score. All exposure measures were divided in quartiles for analysis. A null association was found for breast cancer across all exposure indices, both by county and by ADD. For ovarian cancer, the data suggest an inverse association, with increasing exposure linked to decreasing incidence rates, both at the county and ADD level. The following are the rate ratios (RR) and corresponding 95% confidence intervals, for the summary exposure scores in the three upper quartiles, using the lowest quartile as baseline (RR = 1.0), and the county as the unit of analysis: 1.01 (0.83–1.21), 0.77 (0.66–0.90), and 0.76 (0.65–0.88). Due to the ecologic nature of this study and inherent limitations, it is possible that other factors may be contributing to these findings. Studies using individual-level data are recommended to elucidate the relationships between estrogenic environmental exposures and female reproductive cancers.

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Breast cancer is the most common cancer, other than nonmelanoma skin cancer, and the second most frequent cause of cancer death among women in the United States (ACS 2000). In 2000, approximately 183,000 new cases were expected to be diagnosed, and over 40,000 women were likely to die from this disease. Ovarian cancer, with approximately 23,100 new cases and 14,000 deaths annually, is the gynecologic malignancy with the highest number of deaths in the United States (ACS 2000). Both cancers share some of the same risk factors in women, including a protective role for pregnancy, related to both age at first pregnancy and further reduction in risk with increasing number of pregnancies, and breast-feeding (Whittemore *et al.* 1992; Whittemore 1993; Kelsey *et al.* 1993). Conversely, early age at menarche, late age at menopause, hormone replacement therapy (HRT), and high socioeconomic status increase the risk for both cancers (Riman *et al.* 1998; Kelsey *et al.* 1993; Vogel 2000; Garg *et al.* 1998; Collaborative Group on Hormonal Factors in Breast Cancer 1997; Baquet and Commiskey 2000), whereas prolonged use of clomiphene, a drug used in infertility treatments, appears to increase the risk of ovarian cancer (Rossing *et al.* 1994) and oral contraceptive use seems to decrease the risk (La Vecchia and Franceschi 1999; Purdie *et al.* 1995). In general, lifetime exposure to estrogen increases the likelihood of developing these cancers (Risch 1996; Dowsett 2000). In addition, they share genetic factors, since family history of breast cancer is a risk factor for ovarian cancer (Tonin 2000).

The association observed between both ovarian and breast cancer and endogenous estrogen or hormonally related events led to the hypothesis that exogenous estrogenic compounds in the environment could also have an effect (Davis *et al.* 1993; Gillesby and Zacharewski 1998). For example, the link proposed between breast cancer and organochlorines is based on evidence of their estrogenic activity, considerable use and persistence, and carcinogenicity in animals. Growing concern for the role of environmental estrogenic compounds also comes from studies linking exogenous exposures to chromosomal and specific genetic alterations (Lasky and Silbergeld 1996).

Over the past several decades, there has been a sharp increase in world pesticide production, doubling every 10 years since 1945 (Dich *et al.* 1997). Breast cancer incidence rates in the United States have increased by approximately 1% per year

since 1940 (Laden and Hunter 1998), but only 30–45% of cases in the United States are attributable to the established risk factors mentioned above (Madigan *et al.* 1995; Davis *et al.* 1993). Age-adjusted ovarian cancer mortality rates have changed little in the United States from 1979 to 1995, but rates are increasing in women aged 65 years and older and decreasing in younger women (Oriel *et al.* 1999).

Atrazine [6-chloro-*N*-ethyl-*N′*-(1-methylethyl)-1,3,5-triazine-2,4-diamine], an organochlorine compound, has been widely used as a herbicide in the United States for at least 40 years, mainly in corn production, and it is currently the most commonly used herbicide. With 68–73 million pounds of active ingredient applied in 1995, atrazine is the predominant herbicide used according to pounds applied (Short and Colborn 1999). It is one of the major pesticides found in drinking water, with elevated concentrations found in some water supplies (Taets *et al.* 1998), and detectable amounts measured in many areas of the United States (Short and Colborn 1999).

Although most of the studies of pesticides and cancer have focused on DDT (now banned in the United States), atrazine has been classified as a possible human carcinogen by the International Agency for Research on Cancer (IARC 1999). Animal studies showed that lifetime feeding of atrazine to Sprague-Dawley rats, at a maximum tolerated dose (MTD), lengthened their estrus cycle, increased the number of days in estrus, or, given in conjunction with estrogen, induced an earlier onset of mammary tumors (Wetzel *et al.* 1994). Two epidemiologic studies suggest that atrazine may be carcinogenic to humans. In a case-control study by Donna *et al.* (1989), women with previous exposure to triazine herbicides showed a two- to threefold risk of epithelial ovarian cancer as compared to unexposed women. A recent study conducted in Kentucky suggests a modest association between triazine exposure and an increased risk of breast cancer (Kettles *et al.* 1997).

Atrazine is the main herbicide used in Kentucky (approximately 1 million pounds annually), primarily on corn crops (Department of Entomology 1991). It is usually applied directly onto the soil by a power or tractor pulled sprayer (Collins, personal communication). The current study was designed to expand the previous ecologic study of breast cancer in Kentucky (Kettles *et al.* 1997), investigating the relationship between several indices of atrazine exposure and the incidence of ovarian cancer and breast cancer over a 5-year period.

Materials and Methods

Study Design

An ecologic study was conducted using secondary data to derive measures of environmental exposure to atrazine in Kentucky and to examine the association between these measures and the incidence of ovarian and breast cancer incidence.

Study Population

Kentucky has a population of approximately 3.7 million persons, distributed among 120 counties. Counties are grouped into 15 Area Development Districts (ADDs) (Figure 1), which are public bodies under Kentucky law. The counties in each ADD share common features, characterized by their geography, production, or economic development. For convenience and the sake of brevity, we tabulate the descriptive data by ADD and not by county.

Outcome Measurement

We obtained ovarian and breast cancer age-adjusted incidence rates, aggregated over a five year period: 1993–97, by county and by ADD from the Kentucky Cancer Registry (KCR). The KCR has maintained a statewide registry since 1991, collecting data on all new cancer cases diagnosed throughout the state. It has developed into a reliable and complete population-based cancer registry, with over 99% case ascertainment (North American Association of Central Cancer Registries 2001). To be included in this study, all cases of ovarian and breast cancer had to be newly diagnosed during the study period and to have identified a Kentucky county as the location of usual residence at the time of diagnosis.

Exposure Measurement

For this study, we assumed that the main route of atrazine exposure to the general population was through drinking water. However, due to the limitations in drinking water data mentioned below, we used three sources of data to derive atrazine exposure indices: public drinking water atrazine levels, acreage of corn planted, and atrazine sales in Kentucky.

The Environmental Protection Agency (EPA), in accordance with the Safe Drinking Water Act of 1974, set the maximum contaminant level for atrazine in drinking water at 3 parts per billion (ppb). This federal regulation became effective in 1992, and between 1993–95, the EPA required public water companies to test water samples for atrazine quarterly for 1 year. If atrazine levels were above 1 ppb the water company must continue to monitor the contaminant in their water system (U.S. EPA).

Data on atrazine concentrations in Kentucky's drinking water supplies were provided by the Kentucky Division of Water (DOW), Drinking Water Branch of the Kentucky state government. Data were collected from all public water systems, with information on the name and address of each company, size and location (by county) of the population served, activity status of the company (active/inactive), and primary water source (ground, purchase ground, surface, purchase surface) of each water system, date of sample, and atrazine level measured in μg/L.

The DOW data also contained information on the types of public water systems according to the following classifications: Community Water System (CWS), which supplies water to the same population year-round; Non-transient Non-community Water System (NTNCWS), which supplies water to at least 25 of the same people at least 6 months per year (but not year-round), such as schools, factories, office buildings, and some hospitals; and Transient Non-community Water System (TNCWS), which provides water in such places as gas stations or campgrounds. We examined the mean atrazine level for each type of water system separately, and compared those to the means when all systems were combined. NTNCWS and TNCWS were grouped together into noncommunity water systems (NCWS) for analysis and then compared to the means of the CWSs. Because the differences between the combined means did not differ greatly from the separate CWS and NCWS values, we used the combined atrazine mean figures in our statistical analysis.

Data from the DOW, and the 1990 census were used to determine the extent to which the population in Kentucky, which is largely rural,

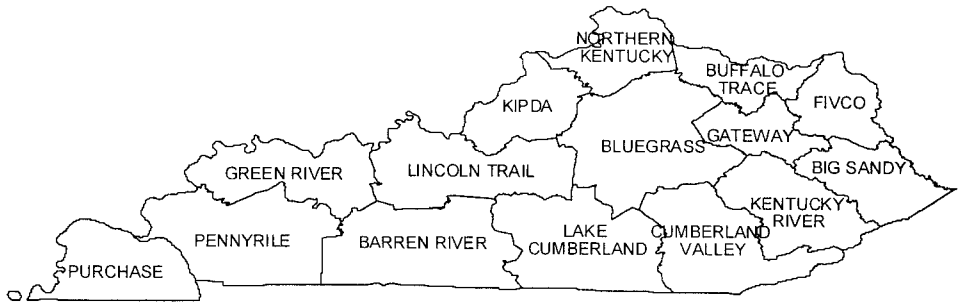

Fig. 1. Kentucky's 120 counties are grouped into 15 area development districts

relies on private wells as their source of drinking water, for which atrazine levels are not available. The results indicated that the public water data missed a substantial proportion of the population, particularly in some areas (as described below). We used two additional, surrogate atrazine indicators to estimate exposure by county and ADD, as a means to overcome this limitation.

According to the Kentucky Division of Pesticides in Frankfort, atrazine accounts for approximately 20–33% of the total pesticide sales in the top 20 counties with the highest sales. Since the EPA considers atrazine a restricted use pesticide (RUP), it can only be purchased and used by certified applicators and the state has a record of these sales. We used data on atrazine sales in 1997 throughout the state, which was available for 109 of the 120 counties. Because atrazine is primarily used to control weeds in the production of corn, we obtained information on the number of acres of corn planted in each of Kentucky's 120 counties for the years 1970, 1980, and 1990 from the Kentucky Department of Agriculture.

Demographic Covariates

We obtained demographic data from the 1990 U.S. Census and aggregated it at the county and ADD level. We used the proportion of the population over 25 years of age with at least 4 years of college education, median household income, and percent black race as socioeconomic indicators. These indicators are shown in Table 1, along with the proportion of the population classified as rural (also from 1990 census).

Statistical Analysis

For each water company, we obtained all the atrazine measurements taken between January 1992 and May 1999 for 117 counties. There was a wide range in the number of atrazine measurements performed by each water company (8–231), as well as in the size of the population it served. We derived a single atrazine water exposure level per water system by averaging all the measurements in the database for each company. To estimate the county or ADD atrazine index, we averaged all these calculated values per company across all the companies in each county or ADD, taking into account the size of the population they served. In this way, we calculated a population-weighted mean atrazine water level for each unit of analysis.

We classified the source of public water into ground water (combining ground and purchased ground) and surface water (combining surface and purchased surface). We calculated weighted means as described above, for surface and ground water separately, and compared the results to the weighted means of all systems combined.

Since a substantial proportion of the population in Kentucky uses private water and there is a wide variation across counties and ADDs, we estimated the proportion of people served by private water supplies in the state. We used data provided by the Kentucky Geological Survey at the University of Kentucky (Carey *et al.* 1993), which was extracted and summarized from the 1990 census and coded by county. We summed across counties for the analysis by ADD.

Due to the large proportion of the population using private water in some areas (see Table 2), we used two other surrogate exposure measures, as explained above. First, we examined the acreage of corn planted in three different time periods: 1970, 1980, and 1990. The acreage of corn planted at the county level was highly correlated over the three decades (1970:80 $r = 0.93$; 1970:90 $r = 0.94$; 1980:90 $r = 0.94$). Considering the usual latency period from time of exposure to development of cancer, we chose to use the 1970 data. We added acres of corn planted per county across all counties in each ADD to get acres of corn by ADD.

Finally, we also used the atrazine sales data. This was obtained from the Department of Agriculture, Division of Pesticides, and was in the form of number of pounds sold per county.

For each of the three sources of exposure (atrazine in water, atrazine sales, and acres of corn) we conducted univariate analysis to determine the quartile distribution. We derived a score for each ADD by averaging the scores of all the counties in each ADD. Finally, we created a summary total composite exposure score combining the scores from the three measures. For each county, we summed across the quartile values of the three exposure variables (corn, sales, water) to give a total score (0–9). In the few cases in which we encountered missing data at the county level for atrazine sales (11 counties) and atrazine in water (3 counties), we imputed the average across all the other counties in each ADD. The total scores for each county were averaged to give a total score for each of the 15 ADDs.

The covariates examined, both at the county and ADD level, included the following: (a) the percentage of black residents, (b) the percentage of those 25 years or older with 4 or more years of college, (c) the percentage of people residing in a rural area, (d) the median household income, and (e) the percentage of people using public water.

For the estimation of the association between atrazine exposure and the risk of ovarian and breast cancer, we employed the Poisson regression method, which allows for the statistical modeling of data when there is a small number of events within strata. Rate ratios (RRs) and 95% confidence intervals were calculated using the PC-SAS software package (SAS Institute 1990). Crude RRs were first calculated for each predictor variable, separately for the two cancers, followed by a multivariate analysis. Due to the high correlation between the education and income variables ($r = 0.92$), we excluded income from the multivariate Poisson models. For the atrazine exposure indices, we performed the analysis for each one separately and finally with the total composite exposure score.

Table 1. Demographic characteristics for ADDs in Kentucky for 1990[a]

ADD	Population	Race (% Black)	College Education (% with ≥4 years)	Median Income ($/Household/year)	% Rural Population
Barren River	222,766	6.2	15.3	20,243	66.1
Big Sandy	165,020	6.2	13.4	16,524	91.4
Bluegrass	590,336	8.3	21.1	25,708	35.2
Buffalo Trace	51,877	2.9	13.5	18,674	80.3
Cumberland Valley	223,024	1.4	12.1	14,664	81.2
Fivco	132,685	0.9	18.3	21,581	55.1
Gateway	66,346	2.4	13.6	17,003	79.3
Green River	199,342	5.1	19.6	23,519	49.6
Kentucky River	123,495	0.6	11.2	14,170	93.4
KIPDA	796,491	14.8	23.8	27,787	15.2
Lake Cumberland	174,283	1.9	12.2	16,087	83.8
Lincoln Trail	219,101	8.0	19.5	22,554	60.7
Northern Kentucky	334,979	1.7	22.1	29,576	27.5
Pennyrile	205,800	12.1	19.3	20,933	58.7
Purchase	181,346	6.1	21.2	21,366	55.7
All Kentucky	3,686,891	5.2	17.1	20,693	62.2

[a] 1990 census data.

Table 2. Characterization of public water usage in Kentucky by ADD

ADD	Total Population Served	Total Companies[a]	% Public[b]	% Surface[c]
Barren River	259,240	19	82.4	99.8
Big Sandy	80,227	40	46.2	94.6
Bluegrass	585,741	36	91.0	99.9
Buffalo Trace	39,714	10	70.3	47.8
Cumberland Valley	169,184	55	64.2	94.0
Fivco	98,231	13	73.1	88.5
Gateway	39,662	10	67.2	98.7
Green River	149,106	26	90.1	49.2
Kentucky River	65,076	14	31.4	79.0
KIPDA	833,321	74	96.4	95.3
Lake Cumberland	134,604	21	63.9	99.7
Lincoln Trail	218,193	34	69.9	94.6
Northern Kentucky	181,428	31	86.0	90.1
Pennyrile	179,967	32	83.4	95.4
Purchase	162,335	53	75.5	29.0
All Kentucky	3,196,029	468	72.7	83.7

[a] DOW data.
[b] Census data.
[c] Proportion of public water from a surface water source (vs. ground).

Results

The demographic characteristics presented in Table 1 show that that the majority of the population in Kentucky is white, with only 5% black overall (county range = 0–25%). The proportion of other minorities was less than 1% in the 1990 census (this may be changing with the increasing influx of Hispanics into Kentucky in the past 10 years). The KIPDA ADD has the highest proportion of blacks (15%), mainly due to their concentration in the larger city of Louisville (Jefferson County, 25%) and surrounding areas. Over 60% of the population of the state is considered rural, but there is substantial variation, from 15% in the KIPDA ADD to 93% in the Kentucky River ADD (county range = 2.8–100%). The proportion of persons over 25 years of age with at least 4 years of college ranged from 7.3% in the Lake Cumberland ADD to 18.2% in the KIPDA ADD (county range = 4.6–30.6%). The median income in Kentucky is quite low, ranking 11th lowest nationwide by state (U.S. Census Bureau 1990). However, there is wide variability across ADDs ($14,170–$29,576) and counties ($11,110–$42,143).

The exposure data is summarized by ADD in Tables 2 and 3. In Table 2, we present the general characteristics of public drinking water sources in Kentucky. There are a total of 468 public water supply companies in Kentucky with atrazine measurements, ranging greatly in the size of the population served (375 to 833,321). Overall, 73% of the population is served by public water supplies, but there is wide variation among counties (10% to 99%) and among ADDs (31% in Kentucky River to 96% in KIPDA). The geographic distribution of these variations agreed with the notion that those who live in urban areas are mostly supplied by public water, whereas predominantly rural areas are mainly dependent on private water sources. Although surface water constitutes the main source of public water as a whole (84%), we observed a wide range at the county level (0.86% to 100%) and at the ADD level (29% in Purchase to almost 100% in the Bluegrass ADD).

The comparison of atrazine levels for surface water and ground water resulted in weighted means for surface water ranging from 0.21 μg/L in the KIPDA ADD to 1.66 μg/L in the Purchase ADD, and for ground water ranging from 0.19 μg/L in the KIPDA ADD to 1.19 μg/L in the Barren River ADD. When all water sources were combined, the weighted means ranged from 0.21 μg/L in the KIPDA ADD to 1.39 μg/L in the Gateway ADD. Because the majority of the public water comes from surface water, the combined means and the surface water weighted means did not differ dramatically, thus we used the combined atrazine mean figures in our statistical analysis.

Table 3 shows the three exposure variables by ADD, with mean levels, individual scores, and composite total scores. Although we did not find any population-weighted atrazine

Table 3. Estimated exposure metrics for atrazine in drinking water, atrazine sales, and acres of corn planted, by ADD

ADD	Water (μ/L)		Sales (Pounds)	Corn Planted (Acres)	Water Score[b]	Sales Score[b]	Corn Score[b]	Total Score[b]
	Mean	Range[a]	Range[a]	Range[a]				
Barren River	0.84	0.06–8.00	20–126,378	48–315	1.4	2.1	2.4	5.9
Big Sandy	1.12	0.05–5.00	340–460	4–19	2.4	0.0	0.0	2.4
Bluegrass	0.45	0.05–20.00	340–10,802	0–131	1.2	1.3	1.2	3.7
Buffalo Trace	0.47	0.05–2.50	133–12,144	5–121	0.8	1.4	1.2	3.4
Cumberland Valley	0.93	0.01–5.00	50–2,765	3–78	1.6	0.8	0.8	3.1
Fivco	0.29	0.04–5.00	260–1,344	10–32	0.6	0.8	0.4	1.8
Gateway	1.39	0.05–5.00	40–3,140	12–64	2.2	0.4	0.6	3.2
Green River	1.13	0.01–8.22	7,341–129,442	71–653	2.6	2.9	2.9	8.3
Kentucky River	0.87	0.02–2.50	65–240	3–25	1.3	0.0	0.1	1.4
KIPDA	0.21	0.05–2.10	400–27,646	25–188	0.6	1.4	1.7	3.7
Lake Cumberland	0.92	0.03–2.50	4,455–13,017	4–139	1.7	1.9	1.9	5.5
Lincoln Trail	1.09	0.15–7.49	6,653–36,053	90–257	1.3	2.5	2.5	6.2
Northern Kentucky	0.78	0.05–2.55	66–2,056	5–72	1.4	0.3	0.5	2.1
Pennyrile	1.20	0.02–23.00	690–114,821	56–543	2.2	2.7	2.4	7.3
Purchase	1.06	0.02–4.00	12,124–97,387	63–244	1.6	2.9	2.5	7.0

[a] Range among counties within each ADD.
[b] Scores for each ADD; see text for explanation fo their calculations.

mean levels (ADD or county-wide) in drinking water to be greater than the MCL of 3 μg/L, 10 of the 15 ADDs did have individual measurements with levels above the MCL. The total score represents the average of the water, corn, and sales scores combined, and ranges from 1.4 to 8.3. As expected, atrazine sales and acres of corn planted were highly correlated ($r = 0.86$). However, the correlation of these two variables with atrazine in public drinking water was weaker (water:corn $r = 0.26$, $p < 0.005$; water:sales $r = 0.30$, $p < 0.001$).

Tables 4 and 5 summarize the Poisson regression results for the univariate analysis of predictor variables as crude RRs with the 1993–97 breast cancer and ovarian cancer incidence rates as the dependent variables, using the county as the unit of analysis. For both breast and ovarian cancer, higher education and income were weakly but positively associated with increased incidence, reaching statistical significance only in some subgroups, mostly in the analysis by county. Atrazine levels in drinking water showed a slight negative tendency associated with a protective effect for breast and ovarian cancer. A similar effect was observed for the groups with the higher percent of rural population. As for race, areas with a larger proportion of black residents were weakly associated with an increased incidence of both cancers at the county level.

In the univariate analysis, we did not find indication of an association or a trend between the atrazine exposure scores and breast cancer incidence. However, our analysis by county showed a decreasing trend in ovarian cancer risk with increasing exposure, across each of the three individual indices (atrazine in water, atrazine sales, and acres of corn planted). This association was statistically significant and strongest for corn.

The multiple regression models were adjusted for race and education, and none of the exposure indices showed any evidence of a positive association with breast or ovarian cancer using the ADD as the unit of analysis (not shown). At the county level, the associations between each of the exposure measures showed a trend of decreasing ovarian cancer risk with increasing exposure, with statistically significant results particularly in the highest exposure quartile, and strongest for corn, and for all three combined. For breast cancer, the only positive association was observed in the highest corn quartile. These regression analyses were repeated for only those counties with a 50% or greater rural population (102 counties), and the results were similar (data not shown).

Discussion

Primary Findings

The results of this ecologic study did not generally support an association between exposure to the herbicide atrazine, as estimated by several county- and ADD-level exposure metrics, and an increased incidence of either breast or ovarian cancer among Kentucky women. However, the data do suggest an inverse association between the exposure indicators and ovarian cancer incidence. The protective association for ovarian cancer is most apparent for the corn score and the total exposure metrics in the county level analyses, while adjusting for other covariates. The breast cancer effect estimates indicate a null association with increasing exposures for all the atrazine exposure metrics.

The inverse association between the total exposure estimate and the corn score with ovarian cancer incidence may be due to certain lifestyle or reproductive factors that are differentially distributed among rural women and influence the risk of ovarian cancer (Duell *et al.* 2000). For example, rural women may have an earlier age at first birth, higher mean number of births, older age at menarche, and younger age at menopause. (Bushy

Table 4. Crude rate ratios (RRs) by quartiles of various exposure metrics, using counties as the unit of analysis

Atrazine Exposure Metrics	1993–97 Breast Cancer Incidence Rates		1993–97 Ovarian Cancer Incidence Rates	
	Crude RR	CI	Crude RR	CI
Water score (μg/L)				
0–0.5	1.00	—	1.00	—
0.5–1.5	0.99	0.95–1.04	0.97	0.84–1.12
1.5–2.5	0.94	0.90–0.99	0.90	0.78–1.04
2.5–3	0.96	0.92–1.01	0.88	0.76–1.01
Sales score (pounds)				
0–0.5	1.00	—	1.00	—
0.5–1.5	0.98	0.94–1.04	1.02	0.89–1.17
1.5–2.5	1.06	1.01–1.11	0.91	0.78–1.04
2.5–3	1.03	0.98–1.09	0.86	0.75–1.00
Corn score (acres)				
0–0.5	1.00	—	1.00	—
0.5–1.5	0.95	0.91–1.00	1.00	0.87–1.15
1.5–2.5	0.99	0.95–1.05	0.91	0.79–1.05
2.5–3	0.96	0.91–1.01	0.85	0.74–0.98
Total score				
0–3	1.00	—	1.00	—
3–4	1.03	0.96–1.10	1.10	0.91–1.31
4–6	0.98	0.93–1.02	0.84	0.73–0.96
6–9	1.01	0.96–1.05	0.85	0.75–0.97
% Public water				
0–55.2	1.00	—	1.00	—
55.2–72.0	1.04	0.99–1.09	1.06	0.92–1.22
72.0–85.9	1.07	1.02–1.13	1.07	0.93–1.24
85.9–99.7	1.14	1.08–1.19	1.09	0.94–1.26
% Rural population[a]				
0–69.1	1.00	—	1.00	—
69.1–99.9	0.92	0.88–0.96	0.86	0.75–0.97
99.9–100	0.85	0.81–0.88	0.77	0.77–0.98
Education (% with 4 or more years college)				
0–6.3	1.00	—	1.00	—
6.3–7.7	1.00	0.95–1.05	1.21	1.05–1.40
7.7–10.3	1.10	1.05–1.16	1.25	1.08–1.45
10.3–30.6	1.16	1.11–1.22	1.20	1.04–1.38
Income (median household/year, in $)				
0–19,171	1.00	—	1.00	—
19,171–23,936	1.06	0.93–1.22	1.04	0.90–1.20
23,936–27,051	1.12	0.98–1.28	1.03	0.89–1.20
27,051–42,143	1.18	1.02–1.36	1.10	0.96–1.27
Race (% black)				
0–0.4	1.00	—	1.00	—
0.4–2.4	1.03	0.98–1.09	1.21	1.04–1.40
2.4–5.7	1.11	1.05–1.17	1.17	1.00–1.40
5.7–24.5	1.06	1.01–1.12	1.14	0.97–1.33

[a] Greater than one-fourth of the counties were 100% rural.
CI = 95% confidence interval.

1993; Duell *et al.* 2000). All these characteristics have been shown to be protective for ovarian cancer. A recent study found that farm women in general may have a reduced risk of breast cancer, although subpopulations with documented pesticide exposure may be at an increased risk (Duell *et al.* 2000). The complex issues of potential confounding between the lifestyle factors related to female reproductive cancers and the presumed increased potential for exposure to agricultural pesticides among rural women is difficult to address at the ecologic level.

Comparison with Other Studies

In a review of eight epidemiologic studies, Neuberger (1996) concluded there was limited support for an association between atrazine exposures and several cancers, and the IARC (1999) concluded that there was inadequate evidence in humans to determine the carcinogenicity of atrazine. The two previous studies that have demonstrated an association between exposure to atrazine and female reproductive cancers have reported only marginally increased risks. The case-control investigation

Table 5. Adjusted RRs[a] for breast and ovarian cancer by different exposure metrics, classified by counties

	1993–97 Breast Cancer Incidence Rates		1993–97 Ovarian Cancer Incidence Rates	
Atrazine Exposure Metrics	RR	CI	RR	CI
Total score				
0–3	1.00	—	1.00	—
3–4	1.03	0.96–1.11	1.01	0.83–1.21
4–6	0.97	0.92–1.02	0.77	0.66–0.90
6–9	0.98	0.93–1.04	0.76	0.65–0.88
Sales score				
0–0.5	1.00	—	1.00	—
0.5–1.5	1.00	0.94–1.05	1.06	0.92–1.22
1.5–2.5	1.07	1.01–1.13	0.86	0.73–1.01
2.5–3	1.04	0.98–1.10	0.80	0.67–0.96
Corn score				
0–0.5	1.00	—	1.00	—
0.5–1.5	0.92	0.87–0.97	0.95	0.82–1.10
1.5–2.5	0.97	0.91–1.02	0.83	0.71–0.97
2.5–3	0.92	0.86–0.98	0.76	0.64–0.90
Water score				
0–0.5	1.00	—	1.00	—
0.5–1.5	1.04	0.99–1.10	0.98	0.85–1.14
1.5–2.5	0.96	0.91–1.01	0.90	0.78–1.04
2.5–3	0.96	0.92–1.01	0.85	0.73–0.98

[a] Controlling for race and education.
CI = 95% confidence intervals.

by Donna *et al.* (1989) found a significant association between users of triazines and ovarian cancer, and the ecologic study by Kettles *et al.* (1997) reported a significant but modest increased risk of breast cancer with a summary indicator of exposure to triazine herbicides.

Our study is most comparable to the investigation by Kettles *et al.* (1997) with respect to its ecologic design and use of the resources of the KCR for the cancer incidence data, aggregated at the level of the county and the ADDs. With counties as the unit of analysis, the Kettles study reported an effect of triazine exposure with the 1993–94 breast cancer rates for the medium and high triazine exposure categories (OR = 1.14 (95% CI: 1.08–1.19) and 1.20 (95% CI: 1.13–1.28), respectively). Our finding of a null association for increasing levels of the total score variable for atrazine exposure and the 5-year (1993–97) breast cancer data is contrary to the modest risk reported in the Kettles study. This suggests, on an ecologic level, that when breast cancer rates were averaged over a 5-year period, the potential small association between atrazine exposure and breast cancer incidence disappeared. We presume that our 5-year, age-adjusted cancer rates (1993–97), for both breast and ovarian cancer are more stable when compared to the analysis of the 2-year rates by Kettles *et al.* (1997).

We conducted our analyses at both the county and the ADD level. Our comparison of the results using county and ADD as units of geographic analysis yielded similar findings for the evaluation of the four exposure metrics for both breast and ovarian cancers. For brevity, we presented the Poisson regression results at the county level, for which our estimates of precision are improved over the analysis with the ADDs. Although county-level rates are less stable than rates at the level of the ADD, the use of the data for 120 counties in the analysis substantially improved model precision and likely reduced geographic misclassification of exposure in comparison to the use of the 15 ADDs in the state. Generally, the recommendation to employ the smallest geographic unit of analysis available when undertaking ecologic studies appears to be confirmed on the basis of considerations of precision and exposure misclassification (Hertz-Picciotto 1998).

Strengths and Limitations

A strength of this study resides in the analysis of the several sources of exposure data and the validation of the several exposure metrics used to examine the association of proxy indicators of atrazine exposure and reproductive cancer risk. Our use of the Kentucky DOW data, atrazine sales data, and agricultural data on acres of corn planted provided three distinct variables for estimating potential exposure to atrazine at the county level.

Initially, we assumed that the most useful exposure indicator for this study would be the estimate derived from the Kentucky DOW data for measured atrazine levels in the public water supply. These data provided relatively comprehensive geographic coverage of the state since atrazine measurements are mandated by law to be conducted on all public water supplies. However, the levels of atrazine detected in the public drinking water supplies were low, with only a few measurements exceeding the MCL of 3 μg/L. Kentucky is heterogeneous with respect to the production of corn, reported use of atrazine, and proportion of the population served by public water drinking supplies, but there were no strong exposure gradients with respect to the measured atrazine levels (μg/L) across the counties or ADDs. At these contaminant levels, ecologic studies

may have limited sensitivity to detect differences in cancer incidence due to variations of atrazine exposure across geographic units (counties), should such an association exist (Morgenstern 1998).

We were able to compare atrazine measurements among public water supply companies in different areas, and also between surface versus ground water sources. We found that the population-weighted, mean contaminant levels for atrazine in ground water were lower than those in surface water. This agrees with previous reports indicating that atrazine is typically found more frequently, and usually at lower concentrations, in ground water than surface water (IARC 1999).

Several issues limit the usefulness of the DOW contaminant database as the sole source of environmental exposure data. First, given the largely rural nature of the state, a considerable proportion of the population does not use public water sources, but relies on private wells in their own homes or farms. The distribution of private water use varies widely by county (0.3% to 89.6%), and also by ADD (3.6% to 68.6%), so that for some areas public water atrazine measurements would not be representative of the atrazine exposures for local residents. Rural private drinking water wells do not have regulatory requirements for water quality monitoring (Smith *et al.* 1999), and no extensive, representative study has been conducted in Kentucky to measure contaminants in private wells across the whole state. Second, in many areas there are numerous, often small water supply companies with water systems that may overlap county boundaries, work, and home locations. Finally, little is known about the temporal changes in contaminant levels in these water supplies.

We endeavored to address the issues of exposure misclassification of atrazine in drinking water in part through the use of the two other exposure proxy measures: acres of corn planted and atrazine sales by geographical location. Both of these measures were quite strongly correlated with each other, as expected. However, the correlation of these two proxy exposure variables with atrazine levels in public drinking water was weak. We expected to find the highest concentrations of the contaminant in the water supplies of the areas where corn is grown, since atrazine is most commonly used as a herbicide in corn production. The weak correlation between corn production and atrazine sales with atrazine levels in the water may be due to several or a combination of factors, including: (1) atrazine may be used for other purposes not routinely reported; (2) atrazine may be purchased in one area, but used in another; (3) the source of the public water in a given county may be purchased from another county; (4) although the half-life of atrazine has been reported to be 60 days or more (Wauchopa et al. 1996), in Kentucky it has been found to be much shorter due to the composition of the acidic soil (Witt, personal communication). This fact, combined with the seasonal application of atrazine, may introduce increased variability in water atrazine measures.

Although we controlled for education and race in the regression analysis, achieving adequate control of covariates within ecologic studies is difficult. Ecological proxies of individual-level covariates may yield different patterns of association for ecologic studies in comparison to individual-level studies (Morgenstern 1995). For example, the variable for race (proportion of the population that is black) does not show the expected protective effect at the ecologic level that it shows for individual level studies, possibly because the race distribution in the population is strongly associated with other socioeconomic indicators (poverty, health services such as screening, smoking patterns, etc.) that are associated with cancer incidence. Second, the usual covariates employed in environmental epidemiologic analyses (average annual income for population, urban/rural, proportion of population of minority race, etc.) tend to be more highly correlated with each other at the ecologic level than at the individual level, making it difficult to separate the effects of these variables statistically (Morgenstern 1995). Finally, the presence of other water contaminants, such as trihalomethanes, which are more common in surface water sources, could potentially act as confounders. However, these water chlorination by-products have generally been found to be carcinogenic in the kidney, liver, and intestines of rats rather than in the reproductive organs (Dunnick and Melnick 1993).

With regards to cancer incidence data, although rates were based on county of residence at diagnosis, the length of time at the current residence for cancer cases is not known, therefore, misclassification of individual cases, which form the numerators of rates, with respect to county of exposure is a possible, although presumed small, source of bias.

Although not the topic of this study, it has been hypothesized that different types of ovarian cancer may have different etiologies. In particular, investigators have postulated that exogenous estrogen compounds may only increase the risk of nonmucinous ovarian tumors (Risch *et al.* 1996). When we stratified our data we found that the proportion of nonmucinous tumors by ADD ranged from 76–92% (data not shown). It is noteworthy that the highest percent of nonmucinous ovarian cancers were found among the highest atrazine-exposed ADDs.

Our study offers insight for the design of individual level studies to address the issue of exposures to triazine herbicides and reproductive cancers. It highlights the complexity of the exposure assessment necessary for such an investigation, which would require detailed data on water consumption patterns for residents, including profile of usual water supply (public/private), sampling of cases and controls from geographic areas with a gradient of exposure, and assessment of the established risk factors related to breast and ovarian cancers. Studies of atrazine exposure from other sources, such as inhalation or dermal exposure during herbicide application, may yield additional information because exposures may be stronger, yet more sporadic or seasonal. Occupational studies among farmers, including the Agricultural Health Study (Alavanja *et al.* 1994) and workers in triazine manufacturing plants, will likely yield further insights regarding the evaluation of the potential human carcinogenicity of the triazine herbicides (Neuberger 1996). Future studies should also examine specific tumor subtypes of each cancer, which may have different etiologies.

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References

Alavanja MCR, Akland G, Baird D, Blair A, Bond A, Dosemeci M, Kamel F, Lewis R, Lubin J, Lynch C, McMaster SB, Moore M, Pennybacker M, Ritz L, Rothman N, Rowland A, Sandler DP, Sinha R, Swanson C, Tarone R, Weinberg C, Zahm SH (1994) Cancer and noncancer risk to women in agriculture and pest control: the Agricultural Health Study. J Occupat Med 36(11): 1247–50

American Cancer Society (2000) Cancer facts and figures—2000. American Cancer Society, Atlanta, GA

Baquet CR, Commiskey P (2000) Socioeconomic factors and breast carcinoma in multicultural women. Cancer Suppl 88(5):1256–1264

Bushy A (1993) Rural women: lifestyle and health status. Nurs Clin N Am 28(1):187–197

Carey DI, Dinger JS, Davidson OB, Sergeant RE, Taraba JL, Elvento TW, Coleman S, Boone R, Knoth LM (1993) Quality of private ground-water supplies in Kentucky. University of Kentucky, Kentucky Geological Survey, Lexington, KY

Collaborative Group on Hormonal Factors in Breast Cancer (1997) Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 350:1047–1059

Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H (1993) Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ Health Perspect 101(5):372–377

Department of Entomology, Cooperative Extension Service, College of Agriculture, University of Kentucky (1991) Kentucky pesticide user practices and alternatives, University of Kentucky, Lexington

Dich J, Zahm SH, Hanberg A, Adami HO (1997) Pesticides and cancer. Cancer Causes Control 8:420–433

Donna A, Crosignani P, Robutti F, Betta PG, Bocca R, Mariani N, Ferrario R, Fissi R, Berrino F (1989) Triazine herbicides and ovarian epithelial neoplasms. Scand J Work Envrion Health 15: 47–53

Dowsett M (2000) Use of risk determinants for different breast cancer prevention strategies. Eur J Cancer 36:1283–1287

Duell EJ, Millikan RC, Savitz DA, Newman B, Smith JC, Schell MJ, Sandler DP (2000) A population-based case-control study of farming and breast cancer in North Carolina. Epidemiology 11(5):523–531

Dunnick JK, Melnick RL (1993) Assessment of the carcinogenic potential of chlorinated water: experimental studies of chlorine, chloramine, and trihalomethanes. J Natl Cancer Inst 85(10):817–822

Garg PP, Kerlikowske K, Subak L, Grady D (1998) Hormone replacement therapy and the risk of epithelial ovarian carcinoma: a meta-analysis. Obstet Gynecol 92:472–479

Gillesby BE, Zacharewski TR (1998) Exoestrogens: mechanisms of action and strategies for identification and assessment. Environ Toxicol Chem 17(1):3–14

Hertz-Picciotto I (1998) Environmental epidemiology. In: Rothman KJ, Greenland S (eds) Modern epidemiology, 2d ed. Lippincott-Raven, Philadelphia, PA, pp 555–583

Howe HL, Chen VW, Hotes JL, Wu XC, Correa CN, Fulton JP (eds) *Cancer in North America, 1994–1998. Volume One: Incidence.* Springfield, IL: North American Association of Central Cancer Registries, April 2001

International Agency for Research on Cancer (IARC) (1999) Evaluating carcinogenic risks in humans. Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. Vol. 73. World Health Organization, Lyon, France, pp. 59–113

Kelsey JL, Gammon MD, John EM (1993) Reproductive factors and breast cancer. Epidemiol Rev 15(1):36–47

Kettles MA, Browning SR, Prince TS, Horstman SW (1997) Triazine herbicide exposure and breast cancer incidence: an ecologic study of Kentucky counties. Environ Health Perspect 105(11):1222–1227

La Vecchia C, Franceschi S (1999) Oral contraceptives and ovarian cancer. Eur J Cancer Prev 8:297–304

Laden F, Hunter DJ (1998) Environmental risk factors and female breast cancer. Annu Rev Public Health 19:101–123

Lasky T, Silbergeld E (1996) p53 mutations associated with breast, colorectal, liver, lung, and ovarian cancers. Environ Health Perspect 104(12):1324–1331

Madigan MP, Ziegler RG, Benichou J, Byrne C, Hoover RN (1995) Proportion of breast cancer cases in the United States explained by well-established risk factors. J Natl Cancer Inst 87(22):1681–1685

Morgenstern H (1998) Ecologic studies. In: Rothman KJ, Greenland S (eds) Modern epidemiology, 2d ed. Lippincott-Raven, Philadelphia, PA, pp.459–480

Morgenstern H (1995) Ecologic studies in epidemiology: concepts, principles, and methods. Annu Rev Public Health 16:61–81

Neuberger JS (1996) Atrazine and/or triazine herbicides exposure and cancer: an epidemiologic review. J Agromed 3(2):9–30

North American Association of Central Cancer Registries (2001)

Oriel KA, Hartenbach EM, Remington PL (1999) Trends in United States ovarian cancer mortality, 1979–1995. Obstet Gynecol 93(1):30–33

Purdie D, Green A, Bain C, Sisking V, Ward B, Hacker N, Quinn M, Wright G, Russell P, Susil B (1995) Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Int J Cancer 62:678–684

Riman T, Persson I, Nilsson S (1998) Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence. Clin Endocrinol 49:695–707

Risch HA (1996) Estrogen replacement therapy and risk of epithelial ovarian cancer. Gynecol Oncol 63:254–257

Risch HA, Marrett LD, Jain M, Howe GR (1996) Differences in risk factors for epithelial ovarian cancer by histologic type; results of a case-control study. Am J Epidemiol 144(4):363–372

Rossing MA, Daling JR, Weiss NS, Moore DE, Self SG (1994) Ovarian tumors in a cohort of infertile women. N Engl J Med 331:771–776

SAS Institute (1990). SAS/Stat user's guide, 6th ed. SAS Institute, Inc., Cary, NC

Short P, Colborn T (1999) Pesticide use in the U.S. and policy implications: a focus on herbicides. Toxicol Ind Health 15:240–275

Smith CN, Payne WR Jr, Pope JD Jr, Winkie JH, Parrish RS (1999) A field study to compare performance of stainless steel research monitoring wells with existing on-farm drinking water wells in measuring pesticide and nitrate concentrations. Chemosphere 38(4):875–889

Taets C, Aref S, Rayburn AL (1998) The clastogenic potential of triazine herbicide combinations found in potable water supplies. Environ Health Perspect 106(4):197– 201

Tonin PN (2000) Genes implicated in hereditary breast cancer syndromes. Semin Surg Oncol 18:281–286

US Census Bureau (1990) 1990 Census lookup. 1990 Census summary tape file 3 (STF3)—all socioeconomic and demographic variables. Available at http://venus.census.gov/cdrom/lookup

U.S. Census Bureau. Median income of households by state 1984–1999. Available at www.census.gov/hhes/income/histinc/h08a.html (accessed 6 November 2000)

U.S. EPA National primary drinking water regulations: consumer fact

sheet on atrazine. Available at www.epa.gov/OGWDW/dwh/c-soc/atrazine.html

Vogel VG (2000) Breast cancer prevention: a review of current evidence. CA Cancer J Clin 50:156–170

Wauchope RD, Buttler TM, Hornsby AG, Augustijn-Beckers PWM, Burt JP (1996) SCS/ARS/CES Pesticide properties database for environmental decisionmaking. Rev Environ Contam Toxicol 123:1–157, 1992.8–21 (as cited in EXTOXNET, Extension Toxicology Network Pesticide Information Profiles, http://ace.orst.edu/cgi-bin/mfs/01/pips/atrazine.htm)

Wetzel LT, Luempert LG III, Breckenridge CB, Tisdel MO, Stevens FT, Thakur AK, Extrom PJ, Eldridge C (1994) Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague-Dawley and Fischer 344 rats. J Toxicol Environ Health 43:169–182

Whittemore AS (1993) Personal characteristics relating to risk of invasive epithelial ovarian cancers in older women in the United States. Cancer 71(2; suppl):558–565

Whittemore AS, Harris R, Itnyre J (1992) Characteristics relating to ovarian cancer risk. Collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Am J Epidemiol 13:1184–1203