



## Pesticide Appliers, Biocides, and Birth Defects in Rural Minnesota

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Earlier studies by our group suggested the possibility that offspring of pesticide appliers might have increased risks of birth anomalies. To evaluate this hypothesis, 4,935 births to 34,772 state-licensed, private pesticide appliers in Minnesota occurring between 1989 and 1992 were linked to the Minnesota state birth registry containing 210,723 live births in this timeframe. The birth defect rate for all birth anomalies was significantly increased in children born to private appliers. Specific birth defect categories, circulatory/respiratory, urogenital, and musculoskeletal/integumental, showed significant increases. For the general population and for appliers, the birth anomaly rate differed by crop-growing region. Western Minnesota, a major wheat, sugar beet, and potato growing region, showed the highest rate of birth anomalies per/1000 live births: 30.0 for private appliers versus 26.9 for the general population of the same region. The lowest rates, 23.7/1000 for private appliers versus 18.3/1000 for the general population, occurred in noncrop regions. The highest frequency of use of chlorophenoxy herbicides and fungicides also occurred in western Minnesota. Births in the general population of western Minnesota showed a significant increase in birth anomalies in the same three birth anomaly categories as appliers and for central nervous system anomalies. This increase was most pronounced for infants conceived in the spring. The seasonal effect did not occur in other regions. The male/female sex ratio for the four birth anomaly categories of interest in areas of high phenoxy herbicide/fungicide use is 2.8 for appliers versus 1.5 for the general population of the same region ( $p = 0.05$ ). In minimal use regions, this ratio is 2.1 for appliers versus 1.7 for the general population. The pattern of excess frequency of birth anomalies by pesticide use, season, and alteration of sex ratio suggests exposure-related effects in appliers and the general population of the crop-growing region of western Minnesota. *Key words:* agriculture, birth defects, data linking, fungicides, herbicides, pesticide appliers. *Environ Health Perspect* 104:394–399 (1996)

During the last 7 years, we have investigated the genotoxic properties of pesticides and their possible relationship to the risk of non-Hodgkin's lymphoma risk. We reported significant increases in the frequency of breakage and chromosome rearrangements in G-banded lymphocytes from pesticide appliers (1,2). Polymerase chain reaction (PCR) studies of rearrangements of the V(J) region of immunoglobulins showed similar results (3). The importance of the chromosome rearrangements lies in the fact that they are long-lived chromosome aberrations and can be associated with phenotypic changes in gene expression. More importantly for the current study, rearrangements at the cellular and molecular levels occurred at the highest frequency in pesticide appliers who treated stored grain.

These interesting findings in somatic cells (lymphocytes) led us to question whether similar effects could occur in pesticide-exposed persons at the germ-cell level expressed as birth anomalies. We also speculated that endocrine disruption by pesticides with androgenic or estrogenic properties might alter the male-to-female sex ratio of the birth anomalies observed (4–6). The present study was performed to determine the relative merits of these hypotheses.

As a first step, we used the state of

Minnesota birth registry and linked the records of all births, including those with birth defects for the years 1989–1992 ( $N = 210,723$ ) to Minnesota Department of Agriculture (MDA) registrants who were private, state-licensed appliers ( $N = 34,772$ ). We also compared birth and birth defects data with pesticide use data (amount of active ingredient) provided by MDA (7) for the major crop-growing regions of Minnesota (wheat, corn/soybeans) and non-crop-growing areas (forest/urban). More detailed study of specific pesticide use was conducted and compared to the birth registry data by season of birth and conception for major anomaly groups (circulatory/respiratory, urogenital, musculoskeletal/integumental, and nervous system). Finally, the male/female sex ratio of birth anomalies was compared (appliers versus nonappliers) within pesticide use groups.

The data reported below suggest that pesticide use by appliers and exposure in the general population are associated with increased birth defects in general, and certain groups of birth defects. Some pesticides, particularly chlorophenoxy herbicides and/or fungicides, were found as a group to be associated with certain types of birth anomalies.

## Materials and Methods

### Databases

**Agricultural pesticide appliers.** Under agreement with MDA, we were provided with a list of all members of the agricultural community who were certified to apply restricted-use pesticides in 1991. Persons who apply restricted-use pesticides are required by law to undergo a licensing procedure for certification (training and examination). Private appliers are certified every 4 years and are licensed to apply restricted-use pesticides to their own land. The database provided by the MDA includes name, complete address, and birth date of all licensees in this category. The private applier database was used for the current study.

**Birth registry.** The Minnesota Center for Health Statistics of the Minnesota State Department of Health (MDH) provided access to all in-wedlock live births recorded in the state for the years 1989 through 1992. Only births to in-state residents were considered in this study. Included in the database were demographic data identifying both parents, county of residence, birth dates of parents and child, pregnancy risk factors, and race. Reports on birth defects identified at birth by health professionals were provided in accord with state statute. Records of birth defects and grouping of birth defect categories follow the 1989 National Centers for Health Statistics revised guidelines (8). Statewide, 10.9% of all of the records in the registry were incomplete and did not include medical supplement data (i.e., information on anomalies). Regional reporting differences

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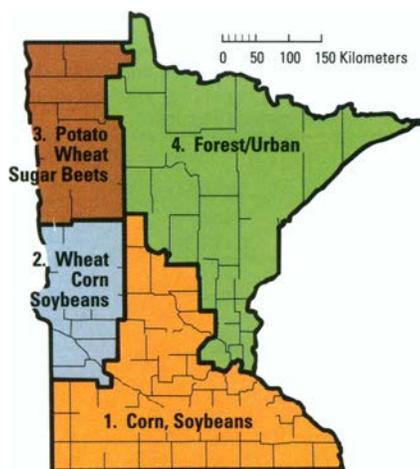
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areas, 8.9% incomplete, wheat/sugar beet/potato growing areas, 5.6% incomplete). Consequently, these births with incomplete medical supplement data were excluded from analysis. To assess this possible confounder, comparisons of pesticide use, applicators, and general population were conducted within each crop-growing and non-crop-growing region. Residents of cities and towns located within non-crop- and crop-growing regions of Minnesota were not excluded from our calculations of birth defect rates.

**Pesticide use survey data.** Access to a statewide pesticide use and crop production survey taken in 1990 and the database derived from the survey (7) was provided by the MDA. The database contains major crop acreage, amount of pesticide used, and treated acreage by county cluster. All Minnesota counties ( $N = 87$ ) in this database were characterized by similar geologic features and similar crops to give 25 distinct units (clusters), each of which was made up of two or more adjoining counties. Northern and northeastern Minnesota is a forest timber and pulp-producing region. Major crop production in southern Minnesota is devoted to corn and soybeans. Western and northwestern Minnesota is a major wheat, potatoes, and sugar beet production region. These more general regional crop divisions, depicted in Figure 1, were provided by the Minnesota Extension Service (9). The pesticide use data reported (MDA) is derived from a total of 12,397 statewide questionnaire respondents selected at random by county cluster from state occupational listings. Stratification of the data into high and low pesticide use by



**Figure 1.** Major crop-growing regions of Minnesota. Each region differs by major crop, geology, weather conditions, and pesticide use pattern. The southern portion of region 2 is a transition zone between wheat and corn/soybean production.

amount of active ingredient (AI) was obtained from the MDA database (7). In the database, quantitative data were available for 12 herbicides by pounds of active ingredient. Minimal use for these herbicides by pounds of active ingredient per county cluster were defined as follows: 2,4-D (CAS no. 94-75-7) <10,000; alachlor (CAS no. 15972-60-8) <10,000; atrazine (CAS no. 1912-24-9) <25,000; bentazon (CAS no. 25057-89-0) <10,000; bromoxynil (CAS no. 1689-84-5) <10,000; cyanazine (CAS no. 21725-46-2) <10,000; dicamba (CAS no. 2463-84-5) <25,000; EPTC (CAS no. 1134-23-2) <10,000; imazethapyr (CAS no. 138261-41-3) <1,000; MCPA (CAS no. 94-74-6) <1,000; metolachlor (CAS no. 51218-45-2) <10,000; and trifluralin (CAS no. 1582-09-8) <50,000. County clusters using more than these amounts of herbicide were defined as high-use areas. In our analyses, the data for 2,4-D and MCPA were pooled because of similarities of chemical structure (both are chlorophenoxy herbicides), spectrum of biocidal activity, and crop use. In this database, quantitative data were not available for insecticide or fungicide use.

**Record linkage and privacy.** The birth record and pesticide applicator database files were translated to the file format native to a relational database management system (Paradox; Borland International, Inc., Scotts Valley, California). Each record was assigned a unique identification number. Confidentiality of personal identifiers was assured by encryption and password protection. A linkage file was constructed containing pairs of identification numbers to represent every birth where the father was also in the pesticide applicator database. Because less than 1% (10) of pesticide applicators were women, females were not considered in the match. The initial linkage was considered valid when all five matching criteria (first name, middle name, last name, birth date, and county) were identical. Further analysis was performed on the remaining data to locate additional matches that would otherwise have been missed (nicknames, data entry errors). Records that matched by four of the five criteria were examined manually for confirmatory information such as address in order to establish the validity of the match. Following the completion of the database linkage, a copy of the birth data field with all personal identifiers removed was prepared and made available for statistical analysis. All work was conducted on a stand-alone personal microcomputer (ZEOS), eliminating potential unauthorized access through network connections. These methodologies were approved by the

Internal Review Board Human Subjects Committee of the University of Minnesota.

### Statistical Analysis

SAS software (SAS Institute, Cary, North Carolina) was used for all statistical analyses. Chi-square tests were used to estimate the statistical significance of observed differences in the proportion of birth anomalies between pesticide applicators and the general population and between populations living in the three identified crop regions. Odds ratios with 95% confidence intervals were developed as estimates of risk. Crude and age-adjusted data were analyzed using the Mantel-Haenszel method (11). Logistic regression (12) was used to estimate the effects of potential pregnancy risk factors analyzed in this study including, but not exclusively, maternal age, education, smoking, alcohol consumption, previous miscarriage, and earlier births with anomalies.

The date of conception was determined from the date of birth and the physician's estimate of length of gestation. Season of birth or conception was designated using the standard calendar date for each of the four seasons. Only births conceived between 1989 and 1991 were included because these data gave complete years within the study timeframe. Data on 1992 conceptions were not available at the time of this study. To examine the frequency of births with anomalies by season of conception or birth, pooled data from major anomaly categories (circulatory/respiratory, urogenital, musculoskeletal/integumental, central nervous system) were combined. The proportion of births with anomalies was compared for 3 consecutive years, 1989-1991. The Mantel-Haenszel method was used to measure overall association (10).

### Results

Table 1 lists the number of births with and without anomalies in private pesticide applicators and in the general population of Minnesota for the years 1989-1992. Only in-wedlock births are represented. Pesticide applicators had significantly more children with an anomaly than did nonapplicators ( $p < 0.001$ ). Further analysis by birth defect category shows that this increase is seen for circulatory/respiratory anomalies ( $p = 0.05$ ); musculoskeletal/integumental anomalies ( $p = 0.02$ ), and urogenital anomalies ( $p = 0.02$ ).

Since most private pesticide applicators are from agricultural regions of the state, we examined the birth defect rate in these regions (Fig. 1, Table 2). Comparison of the rate of birth anomalies among applicators

regions (Fig.1, Table 2). Comparison of the rate of birth anomalies among applicers among the three crop-growing regions and the non-crop-growing region shows that anomalies occur more frequently among private applicers than among the general population from the same region, although the difference is only significant in the corn/soybean-growing region ( $p = 0.02$ ).

In the general population, the pattern of excess of all birth anomalies and in specific birth defect categories in these crop-growing regions (Table 3) is similar to that noted for applicers statewide (Table 1). In specific categories and overall, the greatest birth defect excesses occur in the wheat/sugar beet/potato-growing region. These data show that families residing in predominantly agricultural regions of Minnesota are more likely to have children with birth anomalies.

The volume of pesticide use by pesticide class (herbicides, insecticides, and

fungicides), use of specific pesticides, and method of application differs by crop (13). To explore the possible influence of pesticide use on birth anomalies and to focus our analysis on regional differences in the birth defect rate, specific pesticide use within all crop-growing and non-crop-growing regions was examined. In these analyses, the amount and specific use by county cluster of 12 herbicides were considered in detail for each crop-growing region. Low- and high-level use comparisons were made for each herbicide according to pounds of AI used. Quantities defining low and high use per county cluster were provided by MDA. For example, the use of 2,4-D ranged from <10,000 lbs to >40,000 lbs AI per county cluster. All county clusters with greater than 10,000 lbs AI use were defined as high-use areas. Similar discriminant pesticide-use data was provided for each of the 12 herbicides examined in detail (see Methods). For

other pesticide classes (i.e., insecticides and fungicides), analyses were limited and were conducted according to use by pesticide class. County clusters reporting any use of insecticides or fungicides were compared within the same region to those not reporting use of these products.

Of the herbicides examined, trifluralin showed a modest but significant increase in birth anomalies for the combined births with central nervous system, circulatory/respiratory, urogenital, and musculoskeletal anomalies [odds ratio (OR) = 1.59; 95% CI, 1.39–1.80] and for all anomalies (OR = 1.35; 95% CI, 1.23–1.49). Atrazine use showed a significant increase for all birth anomalies (OR = 1.13; CI, 1.04–1.24) and for combined birth anomalies (OR = 1.33; 95% CI, 1.19–1.49) at only one herbicide use level (>100,000 vs <100,000). At herbicide use levels defined by MDA as high-use areas (> 25,000), atrazine did not show a significant increase in birth anomalies compared to low-use areas. The most consistent results were obtained for 2,4-D and MCPA (Table 4). Since these are both chlorophenoxy herbicides, county clusters were defined as low- and high-use areas based on their combined use. The frequencies of anomalies in high-use areas is significantly increased (OR = 1.86; 95% CI, 1.69–2.05) for combined births with central nervous system, circulatory/respiratory, urogenital, and musculoskeletal anomalies and for all anomalies (OR = 1.51; 95% CI, 1.40–1.62). Table 4 details these data and shows the rate per 1000 births in the general population, with central nervous system, circulatory/respiratory, urogenital, and musculoskeletal anomalies combined by high- and low-use areas within each region; that is, >10,000 lbs AI/county cluster versus

**Table 1.** Live births among private pesticide applicers and general population, 1989–1992: comparison by congenital anomaly category<sup>a</sup>

Anomaly	No. of births with anomaly among		Crude OR (CL)	Age-adjusted OR (CL)
	Pesticide applicers	General population		
Central nervous system	6	242	1.00 (0.44–2.24)	1.10 (0.50–2.40)
Circulatory/respiratory	17	422	1.62 (1.00–2.63)	1.69 (1.04–2.76)
Gastrointestinal	6	175	1.38 (0.61–3.11)	1.74 (0.79–3.83)
Urogenital	20	499	1.61 (1.03–3.52)	1.69 (1.06–2.64)
Musculoskeletal/integumental	30	812	1.49 (1.03–2.14)	
Maternal age <30 <sup>b</sup>	11	517		0.94 (0.52–1.71)
Maternal age >30 <sup>b</sup>	19	295		2.52 (1.58–4.01)
Chromosomal	8	366	0.88 (0.44–1.77)	1.06 (0.53–2.10)
Other	48	1485	1.30 (0.97–1.74)	
Maternal age <35 <sup>b</sup>	36	1321		1.12 (0.80–1.57)
Maternal age >35 <sup>b</sup>	12	164		2.95 (1.63–5.34)
All births with anomalies	125	3666	1.37 (1.15–1.64)	1.41 (1.18–1.69)
Normal births	4456	179,265		

<sup>a</sup>The crude and age-adjusted odds ratios (OR) and confidence limits (CL) for each major birth anomaly category are listed. Pesticide applicers have significantly elevated risks for having children with circulatory/respiratory, urogenital, and musculoskeletal/integumental anomalies. The birth anomaly reporting categories follow the 1989 DHHS guidelines (8).

<sup>b</sup>Maternal age strata used for adjusted ORs are: <25 years, 25–29 years, 20–34 years, and >35 years. Because four maternal age strata for musculoskeletal/integumental and other anomaly categories were not homogeneous, the data were analyzed as shown. Live births for maternal age <18 years were excluded.

**Table 3.** Live births with anomalies in the general population by crop region<sup>a</sup>

Anomaly category	OR compared to non-crop regions (CL)	
	Corn/soybeans	Wheat/sugar beets/potatoes
Central nervous system	1.42 (1.09–1.86)	1.49 (0.92–2.40)
Circulatory/respiratory	1.43 (1.17–1.76)	1.90 (1.37–2.63)
Gastrointestinal	1.09 (0.79–1.49)	1.41 (0.81–2.54)
Urogenital	1.56 (1.29–1.89)	2.25 (1.67–3.03)
Musculoskeletal/integumental	1.36 (1.18–1.58)	1.75 (1.37–2.22)
Chromosomal	0.94 (0.87–1.08)	0.89 (0.57–1.40)
Other	0.97 (0.87–1.08)	1.32 (1.10–1.59)
All births with anomalies	1.16 (1.08–1.24)	1.48 (1.31–1.66)

<sup>a</sup>Odds ratios (OR) are adjusted for maternal age (age strata: <25, 25–29, 30–34, >35 years). Live births for maternal age <18 are excluded. The rate of birth of anomalies in major crop-growing regions is significantly increased compared to the forest/urban region (control). Offspring born to pesticide applicers are excluded.

**Table 2.** Proportion of births with congenital anomalies, by crop region, for private pesticide applicers and the general population

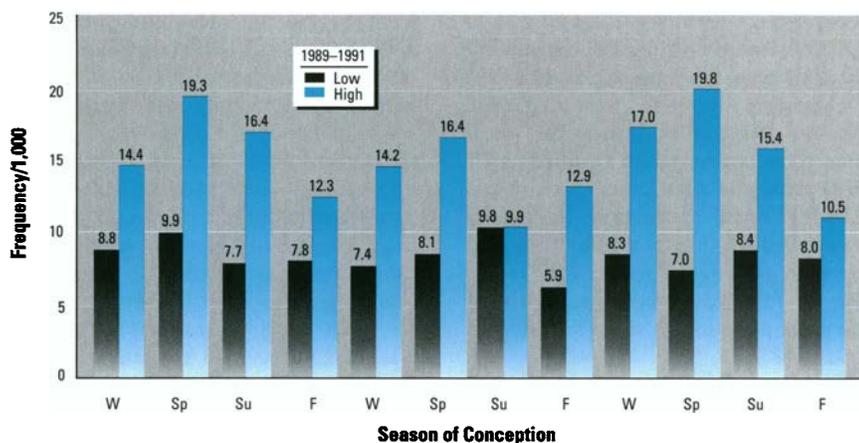
Crop region <sup>a</sup>	No. of normal live births	Births with anomalies	
		No.	Per 1000 live births
<b>1: Corn/soybeans</b>			
General population	68,493	1493	21.3
Pesticide applicers	3379	93	26.8
<b>2, 3: Wheat, corn, soybeans, potatoes, sugar beets</b>			
General population	12,462	344	26.9
Pesticide applicers	874	27	30.0
<b>4: Urban, forest</b>			
General population	98,848	1852	18.3
Pesticide applicers	206	5	23.7

<sup>a</sup>The rate of anomalies/1000 live births is significantly increased in regions 1–3 for the general population and for applicers residing in the same region compared to region 4, a non-crop region. See text for details.

**Table 4.** Frequency per 1000 births of major anomalies for the general population, by region and reported use of chlorophenoxy herbicides/fungicides<sup>a</sup>

Region	Use of chlorophenoxy herbicides/fungicides	Births with anomaly /1000 (totals)	95% CI
Corn/soybeans	Low	9.8 (464/47,506)	8.9–10.7
	High	16.4 (368/22,480)	14.8–18.1
Wheat/corn	Low	—	—
	High	15.2(85/5580)	12.3–18.9
Wheat/sugar beets/potatoes	Low	—	—
	High	15.2 (110/7226)	12.6–18.4
Urban/forest	Low	8.0 (777/97,227)	7.4–8.6
	High	14.7 (51/3473)	11.1–19.4

<sup>a</sup>Major anomalies are central nervous system, circulatory/respiratory, urogenital, and musculoskeletal/integumental. Within each region, based on pesticide use survey information (4), areas of chlorophenoxy herbicide/fungicide use and minimal use were defined. For the general population of each crop-growing region with >10,000 lbs active ingredient chlorophenoxy herbicide/fungicide use per county cluster (high use), the anomaly rate is significantly increased ( $p < 0.001$ ) compared to minimal (low)-use areas.

**Figure 2.** Season of conception of births with central nervous system, urogenital, circulatory/respiratory, or musculoskeletal anomalies, in the general population and chlorophenoxy herbicide/fungicide use. For all years combined, the frequency of anomalies with conceptions in spring or birth in winter is significantly increased ( $p < 0.01$ ), suggesting an exposure-related effect.

use. To test chlorophenoxy herbicide use variability, the 1990 state-sponsored pesticide survey data used in the present work was compared (7) with earlier (1987; data not shown), more detailed federally sponsored pesticide use data (14). Of the 10 county clusters reporting use of more than 40,000 lbs (AI) chlorophenoxy herbicide in the 1990 data, 9/10 showed more than 40,000 lbs (AI) use in the 1987 report. These data suggest year-to-year consistency in chlorophenoxy herbicide use and in reporting among county clusters with the greatest use of these herbicides by amount of active ingredient. Based on the earlier, more quantitative data, six out of seven counties with greatest chlorophenoxy herbicide usage by amount of AI also use fungicides most frequently.

The major use of chlorophenoxy herbicides and almost the entire use of fungicides occurs in the wheat/sugar beet/potato-growing regions of western Minnesota. Application of chlorophenoxy herbicides routinely occurs in the spring; fungicides

are applied as needed in the spring and at later times during the crop-growing season.

As an initial test of the hypothesis that the birth defects observed in the general population could be in part related to chlorophenoxy herbicide and/or fungicide use, the seasonal occurrence of birth anomalies was investigated (Fig. 2) for birth defect categories that were increased in areas of high use of these pesticides.

The data show that in regions where chlorophenoxy herbicides and/or fungicides are frequently used, infants conceived in spring show a significant increase in birth defects compared to infants conceived in the other seasons (OR = 1.36; CI, 1.10–1.69). Results were similar when season of birth was considered (OR for births taking place in winter is 1.33; CI, 1.11–1.59). This effect was not observed for births in regions with low or no reported use of chlorophenoxy herbicides/fungicides. Because of the low number of applicators, seasonality of birth defects could not be tested in this group.

**Table 5.** Proportion of congenital anomalies by sex: progeny of applicators and chlorophenoxy herbicide/fungicide use

	Ratio	No. of male/female births
Normal births	1.05	94,449/89,813
Births with any anomaly	1.38	2210/1604
Births with major anomalies vs. high chlorophenoxy herbicide/fungicide use <sup>b</sup>		
Applicators	2.77	36/13
General population	1.55	373/241
Births with major anomalies vs. low chlorophenoxy herbicide/fungicide use <sup>b</sup>		
Applicators	2.14	15/7
General population	1.72	785/456

<sup>a</sup>In the general population, anomalies are more frequent among male offspring than among female offspring. The applicator group has significantly more male children with anomalies. In regions with high chlorophenoxy herbicide/fungicide use, applicators fathered more male children with anomalies ( $p = 0.05$ ) than the general population in the same region. The increase was not significant in low-use regions.

<sup>b</sup>Major anomalies are central nervous system, circulatory/respiratory, urogenital, and musculoskeletal/integumental.

Birth anomalies in males were more common than in females: 22.6/1000 versus 17.5/1000 ( $p = 0.001$ ) in the general population and 33.3/1000 versus 21.0/1000 ( $p = 0.01$ ) in pesticide applicators, with the rate in male offspring in pesticide applicators being significantly higher (33.3/1000 vs 22.6/1000) than in the general population ( $p = 0.001$ ).

The male-to-female (M/F) ratio of live births is another way to express gender difference (Table 5). For normal births, the ratio is generally reported between 1.04 and 1.07, with slightly more males than females (15). For infants with congenital anomalies, reports detailing the sex ratio of all anomalies are few, but these reported data suggest they predominantly occur in males (16–18). The M/F sex ratio was 1.05 for normal births and 1.38 for births with anomalies. When only births with central nervous system, circulatory/respiratory, urogenital, and musculoskeletal/integumental anomalies are considered, the M/F ratio in county clusters with high chlorophenoxy herbicide/fungicide use is 2.8 for applicators and 1.5 for the general population of the same area ( $p = 0.05$ ). In county clusters with low chlorophenoxy herbicide/fungicide use, the M/F ratio is 2.1 for applicators and 1.7 for the general population.

Using logistic regression to model the probability of births with central nervous system, circulatory/respiratory, urogenital,

probability of births with central nervous system, circulatory/respiratory, urogenital, and musculoskeletal/integumental anomalies with adjustments for known pregnancy risks factors (e.g., maternal age, smoking, alcohol consumption, education, previous miscarriage), the ORs and 95% CIs for risk factors analyzed in this study were: 1.57 (1.22–2.01) for private applicators compared to nonapplicators; 1.62 (1.47–1.78) for male gender compared to female gender; 1.15 (1.03–1.28) for births conceived in spring compared to births conceived in summer, fall, or winter; 1.85 (1.68–2.04) for being a resident of counties with frequent chlorophenoxy herbicide/fungicide use compared to residents of counties with infrequent use of these pesticides. When both applicator status in high/low chlorophenoxy herbicide/fungicide use areas are considered in the same model, the ORs are: 1 (referent group) for the general population living in low pesticide use areas; 1.66 (1.08–2.05) for pesticide applicators residing in the same low-use areas; 1.86 (1.68–2.05) for the general population living in high chlorophenoxy herbicide/fungicide use areas; 1.96 (1.45–2.66) for private applicators residing in the same high-use areas.

## Discussion

The research reported here is an initial step in the evaluation of the possible relationships between the frequency of birth anomalies and pesticide use. These data are similar in some respects to studies from Iowa, Nebraska, and Colorado (18–20). In the Iowa study (19), an association between well water contamination with the herbicide atrazine and excess cardiovascular, urogenital, and limb reduction defects was noted. In the Nebraska study, atrazine in the water supply was associated with excess syndactyly and limb reduction defects as well (20). A more recent Colorado study (21) reported excess urogenital defects in regions with high insecticide use; limb reduction defects were associated with high wheat production; patent ductus/coarctation of the aorta was associated with high levels of pesticide use. Fungicide use was associated with excess chromosome anomalies.

These studies examining the frequencies of birth anomalies in rural agricultural populations from the Midwest grain and corn/soybean regions show that urogenital anomalies and limb reduction deformities are more common in areas where agricultural work is pervasive. In our work, specific excesses of urogenital and musculoskeletal defects (including limb reduction defects and polydactyly/syndactyly/adactyly) were associated with agricultural work

by pesticide applicators, consistent with earlier reports.

Because of the unique geography, geologic history, and climate of different parts of the state, crop production is well defined within specific regions of Minnesota. This allowed us to examine the influence of major crop production on the frequency of birth anomalies. In western Minnesota (regions 2 and 3), spring wheat production predominates (approximately 2.1 million acres), and 2.6% of the live births in this region showed anomalies at birth, more than any region examined (region 1, 2.1%; region 4, 1.8%). In the production of spring wheat, about 90% of the acreage is treated with herbicide. In 1991, more than 70% of the treated acreage was treated with chlorophenoxy herbicides (2,4-D and or MCPA), accounting for approximately 90% of state use (9). Approximately 6% of the wheat crop was treated with fungicides. In the northwestern portion of the wheat region, there is also potato (approximately 53,000 acres) and sugar beet (approximately 168,000 acres) production. Herbicides were applied to 48% of potato acres and more than 60% were treated with fungicides. In 1991, approximately 80% of sugar beet production was treated with fungicide (22).

With regard to biologic plausibility, epidemiologic studies of chlorophenoxy herbicides (23,24) in general do not suggest an association between use of 2,4-D and increased frequency of birth anomalies. Animal studies reviewed by Stevens and Sumner (25) do not indicate that 2,4-D or its analogues are biologically significant teratogens. On the other hand, in a 1993 review conducted by Schardein (26), several animal studies cited indicate that 2,4-D and its analogues can be teratogenic. However, both reviews suggest that a major suspect variable in these animal studies centers on 2,4,5-T and possible dioxin contamination. For 2,4-D, there is limited evidence for genotoxicity in short-term bioassays (27). Synergistic interaction with other biologically and/or chemically active agents may be possible. Animal studies show that the mixture of picloram and 2,4-D, a commonly used commercial product (Tordon 202c), administered to male rodents in drinking water before female conceptions increased the incidence of malformed fetuses (28). Administration of the chemical mixture to females before conception and during the pregnancy produced similar results (29). In other work, administration of 2,4-D in drinking water enhanced viral-induced leukemia in mice (30). Our present data dealing with fungicides and chlorophenoxy herbicides do not exclude

this possibility.

With regard to fertility, a recent clinical epidemiologic study of 2,4-D reports that the herbicide may be spermatotoxic in applicators (31). Along these same lines, preliminary data gathered by our laboratory (unpublished) in regard to birth rate indicate that in the 1990 census year, the frequency of births among applicators (ages 15–44) in the five counties with the highest 2,4-D herbicide/fungicide use was approximately half that of the general population (males ages 15–44) living in the same five-county area ( $p < 0.001$ ).

Fungicide use and exposure are difficult to estimate. In 1990, more than 70% of fungicide applications were made through aerial application methods, with wheat receiving the largest percentage of aerially applied fungicide (7). In the production of wheat, propiconazole (Tilt) was the most commonly used fungicide (nearly 90%), while triphenyl tin (Supertin) was commonly used on sugar beets, and Mancozeb on potatoes. Seed corn, wheat, and potatoes are commonly treated with fungicide (e.g., Captan and Maneb). Use of fungicide-treated seeds in crop production occurs throughout Minnesota.

We found no human studies in the literature that focus on the potential reproductive and teratogenic effects of fungicides. Animal studies show that a number of fungicides have teratogenic potential (32–34) in various rodent species including the fungicides Captan (CAS no. 133-06-2), Mancozeb (CAS no. 8018-01-7), and Maneb (CAS no. 12427-38-2). Propiconazole (CAS No. 60207-90-1) was noted to impair limb ossification at high dose levels in the developing animal fetus (35). Triphenyltin (CAS no. 76-87-9) does not appear to be teratogenic (36), although a congener, tributyltin, does show some teratogenic potential *in vitro* (37) and mutagenicity *in vitro* (38). Other developmental effects of triphenyltin have been reported (39), including effects on spermatogenesis (40).

This study found that the rate of birth defects is increased in: 1) offspring born to licensed private applicators; 2) offspring born to the general population residing in high-use chlorophenoxy herbicide/fungicide regions; and 3) infants conceived in spring. In addition, shifts in the male/female sex ratio of offspring with anomalies were observed. All these findings suggest exposure-related effects.

Alteration in sex ratio of normal live births to fathers employed in pesticide application, deep sea divers, and carbon setters have been reported (41). Preliminary data assembled by H.S. James (unpub-

lished) suggest differences in testosterone levels or gonadotropin levels may be related and affect sex ratio. Of the herbicides showing significant associations with excess birth anomalies in this study, 2,4-D and Trifluralin are endocrine disruptors (42). Of the fungicides mentioned in connection with this study, and which as a group demonstrate excess frequency of anomalies, Mancozeb, Maneb, and tributyltin are endocrine disruptors (42).

The present data and those reported by other investigators in the midwestern agricultural region do signify a clear-cut need for comprehensive examination of the health issues involved.

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