Birth Malformations and Other Adverse Perinatal Outcomes in Four U.S. Wheat-Producing States

Dina M. Schreinemachers

National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

Chlorophenoxy herbicides are widely used in the United States and Western Europe for broadleaf weed control in grain farming and park maintenance. Most of the spring and durum wheat produced in the United States is grown in Minnesota, Montana, North Dakota, and South Dakota, with more than 85% of the acreage treated with chlorophenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA). Rates of adverse birth outcomes in rural, agricultural counties of these states during 1995–1997 were studied by comparing counties with a high proportion of wheat acreage and those with a lower proportion. Information routinely collected and made available by federal agencies was used for this ecologic study. Significant increases in birth malformations were observed for the circulatory/respiratory category for combined sexes [odds ratio (OR) = 1.65; 95% confidence interval (CI), 1.07–2.55]. A stronger effect was observed for the subcategory, which excluded heart malformations (OR = 2.03; 95% CI, 1.14–3.59). In addition, infants conceived during April–June—the time of herbicide application—had an increased chance of being diagnosed with circulatory/respiratory (excluding heart) malformations compared with births conceived during other months of the year (OR = 1.75; 95% CI, 1.09–2.80). Musculoskeletal/integumental anomalies increased for combined sexes in the high-wheat counties (OR = 1.50; 95% CI, 1.06–2.12). Infant death from congenital anomalies significantly increased in high-wheat counties for males (OR = 2.66; 95% CI, 1.52–4.65) but not for females (OR = 0.48; 95% CI, 0.20–1.15). These results are especially of concern because of widespread use of chlorophenoxy herbicides.

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Chlorophenoxy herbicides, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), used since World War II, are widely applied for broadleaf weed control in wheat farming and maintenance of rights-of-way, parks, and home lawns (Short and Colborn 1999). As early as the 1970s and 1980s, Swedish investigators reported increased cancer risks in association with chlorophenoxy acids, especially for soft tissue sarcoma and malignant lymphoma (Axelson and Sundell 1974; Axelson et al. 1980; Eriksson et al. 1981; Hardell 1981; Hardell and Eriksson 1988; Hardell and Sandström 1979; Hardell et al. 1981; Persson et al. 1989; Wingren et al. 1990). Increased numbers of soft-tissue sarcoma were reported for exposed Italian female rice weeders (Vineis et al. 1986). Studies in the United States and Canada confirmed the association with non-Hodgkin lymphoma (Hoar et al. 1986; McDuffie et al. 2002; Woods et al. 1987; Zahm et al. 1990). Dose–response relationships with herbicide-sprayed acreage (mostly chlorophenoxy herbicides) were reported for mortality from non-Hodgkin lymphoma (Wigle et al. 1990) and prostate cancer (Morrison et al. 1993). Short-term immunosuppression, possibly related to cancer etiology, was observed in Italian farmers after application of chlorophenoxy herbicides (Faustini et al. 1996). The lymphocyte replicative index, a measure of cell division kinetics, was increased among applicators exposed solely to 2,4-D over 3 months (Holland et al. 2002).

A recent ecologic study investigated associations between cancer mortality and wheat acreage in agricultural counties of Minnesota, Montana, North Dakota, and South Dakota, where most of the spring and durum wheat produced by the United States is grown (Schreinemachers 2000). Because chlorophenoxy herbicides were used predominantly on wheat, wheat acreage per county was used as a surrogate for chlorophenoxy herbicide exposure. With increasing wheat acreage per county, increasing cancer mortality rates were observed for the stomach, rectum, pancreas, larynx, cervix, ovary, prostate, thyroid, bone, brain, leukemia, eye, nasal cavity, and oral cavity. Another ecologic study, investigating 1989–1992 Minnesota live births, observed increased rates of birth malformations in western Minnesota, where chlorophenoxy herbicides are applied to wheat (Garry et al. 1996). The comparison region was the urban/forested region of the state. This increase was observed not only among private pesticide applicators but also among the general population, suggesting nonoccupational exposure. Rates were highest for births conceived in the spring, peak time of herbicide application.

Both occupational and nonoccupational exposure to chlorophenoxy herbicides can occur through inhalation, ingestion, or dermal contact. 2,4-D has been measured in the urine of children living near a herbicide manufacturing plant and in the urine of adults exposed to recently sprayed turf (Harris and Solomon 1992; Hill et al. 1989). Detectable levels of 2,4-D were measured in semen of recently exposed farmers (Arbuckle et al. 1999b). 2,4-D was detected in indoor air and on surfaces inside homes after lawn application, with indoor activities, children's play, and pets resuspending indoor dust containing 2,4-D (Nishioka et al. 2001). Indoor airborne 2,4-D was associated with inspirable particles. Based on an experimental model, low levels of 2,4-D were expected to be found in residential carpet dust 1 year after turf application (Nishioka et al. 1996). A recent study on pesticide exposure to agricultural workers and their children observed pesticide metabolites in the children's urine (Curl et al. 2002). Although that study did not include 2,4-D, the results pointed to the possibility of a "take-home" exposure pathway. Atmospheric measurements of 2,4-D indicate drift beyond the target area and presence beyond time of application, depending on meteorologic conditions (Larney et al. 1999; Renne and Wolf 1979; Waite et al. 2002). Concentration of 2,4-D in rain has been known to exceed maximum contaminant levels occasionally [U.S. Geological Survey (USGS) 1997]. 2,4-D has been observed in urban and agricultural streams and rivers (USGS 2001).

The ecologic study presented here is based on results of the two previous ecologic studies, which indicated increased rates for two distinct health end points—birth malformations

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Address correspondence to D.M. Schreinemachers, Epidemiology and Biomarkers Branch, Human Studies Division, NHEERL, U.S. EPA, MD 58A, Research Triangle Park, NC 27711 USA. Telephone: (919) 966-5875. Fax: (919) 966-7584. E-mail: schreinemachers.dina@epa.gov

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and cancer mortality—in counties where wheat is the major crop and exposure of residents to chlorophenoxy herbicides is likely (Garry et al. 1996; Schreinemachers 2000). Birth malformations among 1995–1997 births in agricultural, rural counties of Minnesota, Montana, North Dakota, and South Dakota were investigated, comparing high-wheat to low-wheat counties. The purpose of this exploratory study is to identify a potential, regional health hazard. Results should be viewed in this light.

Materials and Methods

Information on newborns and infants for 1995–1997 births was obtained from the Linked Birth and Infant Death files, National Center for Health Statistics (NCHS 1995, 1996, 1997). Agricultural information on crop acreage by state and county and herbicide use by state was obtained from the U.S. Department of Agriculture 1992 census (USDA 1992a, 1992b).

To reflect populations more likely exposed to agricultural pesticides than urban populations, counties in Minnesota, Montana, North Dakota, and South Dakota were selected if at least 50% of the county's population was rural and if at least 20% of the county's land was dedicated to cropland. By selecting counties from these four states, a wide range for the percentage of land dedicated to wheat was obtained. A county was assigned to either the low-wheat or high-wheat group depending on its percentage of wheat acreage with respect to the median of all selected counties. Wheat acreage was used as a surrogate measure for exposure to chlorophenoxy herbicides.

White, singleton births to mothers 18 or more years old were selected if the birth's county of residence was included in the study. The assumption was that county of birth would be the same as the county where the newborn was conceived and the parents lived during pregnancy. Only white, singleton births were included, thereby preventing unreliable results for race (only 18% of all births were to nonwhite parents) and excluding malformed or preterm births due to multiple gestations. The proportion of the following outcome variables in combined high-wheat counties was studied with low-wheat counties as the referent group: *a*) malformations diagnosed at birth; *b*) preterm birth, defined as gestational age < 37 weeks; *c*) small for gestational age (SGA), defined as birth weight below the sex-specific 10th percentile for California white, non-Spanish singleton births for given gestational age (Williams et al. 1982); and *d*) infant death from birth malformations. Malformations were investigated as presented on the birth certificates and in combination with other malformations based on organ system classifications.

Generalized estimating equations methodology using logistic regression was used to estimate the effects on perinatal outcomes in high-wheat counties compared with low-wheat counties (Proc Genmod, SAS/STAT software; SAS Institute 2001). This method accounted for the potential correlation between observations within a county. Odds ratios (ORs; the odds of the effect among the exposed, divided by the odds of the effect among the unexposed), and 95% confidence intervals (CIs) were used to compare frequencies of perinatal outcomes between high-wheat and low-wheat counties. Models were run for combined and single categories of birth outcomes, and for combined and single sexes, provided at least five observations were available for the anomaly category. If more than five observations per exposure group were available for combined sexes but not for one of the sexes, the analysis was conducted for combined males and females only, to prevent small-sample problems. An exception was made for urogenital malformations, which were presented for boys only, because they contributed most of the observations.

Covariate adjustment was performed only if an abnormal birth event was significantly increased in high-wheat counties compared with low-wheat counties. The following covariates were used: maternal age (≥ 35 vs. < 35 years); maternal education (less than high school vs. at least high school); marital status (not married vs. married); parity (first birth vs. second or higher birth); prenatal care (prenatal care in second or third trimester or no prenatal care vs. prenatal care in first trimester); previous preterm or SGA birth (yes vs. no); tobacco use during pregnancy (yes vs. no); alcohol use during pregnancy (yes vs. no); sex of child (male vs. female); time of conception [conception during April through June (time of herbicide application) vs. conception during other months]. Models were run only if at least five observations per covariate were available in each exposure group. A covariate with a p-value ≤ 0.1 for either the male, female, or combined male–female analyses was retained for the final set of covariates for all three analyses, male, female, and combined male–female.

Results

From among the 262 counties in Minnesota, Montana, North Dakota, and South Dakota, 147 agricultural counties with a mostly rural population were selected for the low-wheat ($n = 73$) and high-wheat ($n = 74$) groups (Table 1). The major field crops spring and durum wheat, corn, and soybeans were heavily treated with herbicides (USDA 1992b). Chlorophenoxy herbicides (2,4-D and MCPA) were applied predominantly to spring and durum wheat (88% of acreage) but also to some of the corn acreage (13%) and soybean acreage (4%). Dicamba (3,6-dichloro-2-methoxybenzoic acid), with similar chemical structure and functional groups, was applied to spring and durum wheat (30% of acreage) and corn (48% of acreage), with corn receiving nearly a five-fold higher concentration. The other major herbicide used on corn was atrazine (33% of acreage). Soybeans were mostly treated with imazethapyr (70% of acreage) and trifluralin (47% of acreage). Some of the durum wheat, representing 3% of the total spring and durum wheat acreage, was also treated with trifluralin. Because among herbicides applied to spring and durum wheat, chlorophenoxy herbicides were the most predominant, and other crops were mostly treated with other herbicides, wheat acreage per county was used as a surrogate measure for environmental exposure to chlorophenoxy herbicides. Wheat acreage in 1992 was highly correlated with 1997 acreage (Spearman rank correlation = 0.99). The 1992 acreage was chosen as an estimate for the 1995–1997 time frame of this study.

The number of births during 1995–1997 in the selected counties was 43,634, including 51.4% males, 7.0% premature births, 4.7% SGA births, and 1.9% births diagnosed with malformations. Most births took place in a hospital (99.2%), and most were attended by a physician (95.4%). Absence or presence of malformations at birth was not confirmed on 2% of the birth certificates. Missing information for preterm and SGA births was less than 1%. The covariates sex, maternal age, and marital status were available for all births. Missing percentages for the other covariates were as follows: maternal education, 0.6%;

Table 1. Characteristics of counties selected for this study.

Characteristic	Low wheat	High wheat
Selection criteria		
Rural population (%)	≥ 50	≥ 50
Crop land (%)	≥ 20	≥ 20
Wheat acreage (%)	< 8.6	≥ 8.6
Number of counties		
Minnesota	39	11
Montana	7	9
North Dakota	1	38
South Dakota	26	16
Total	73	74
Combined counties		
Total land ares (acres × 10^6)	46.1	58.3
Agricultural land use (acres × 10^6)		
Total cropland	21.0	34.7
Wheat (for grain)	2.0	12.3
Corn (for grain)	5.4	1.5
Soybeans (for grain)	4.1	1.5
Barley (for grain)	0.4	2.5
Oats (for grain)	0.6	0.6
Potatoes	0.0	0.1
Sugar beets (for sugar)	0.1	0.3
Hay—alfalfa	2.6	2.9
1990 population × 10^3	1,019	421
Rural population (%)	74.2	91.1
Farm population (%)	15.3	19.2

parity, 0.2%; prenatal care, 2.0%; previous preterm or SGA birth, 1.9%; smoking or alcohol use during pregnancy, 15%. Covariate information specific to the low-wheat and high-wheat counties is presented in Table 2.

ORs comparing birth malformations and other prenatal outcomes in high-wheat counties with those in low-wheat counties are presented in Table 3. Anomalies available from birth records were analyzed as single categories, provided enough data were available, and as aggregate categories. Based on the 1989 revision of the U.S. Standard Certificate of Live Birth classification scheme (NCHS 1998), the following categories were included: all central nervous system anomalies—anencephalus, spina bifida/meningocele, hydrocephalus, microcephalus, other central nervous system anomalies; all circulatory/respiratory anomalies—heart malformations, other circulatory/respiratory anomalies; all digestive system anomalies—cleft lip/palate, rectal atresia/stenosis, tracheo-esophageal fistula, omphalocele, other gastrointestinal anomalies; all urogenital anomalies—genital malformations, renal agenesis, other urogenital anomalies; all musculoskeletal/integumental anomalies—poly-/syn-/adactyly, clubfoot, diaphragmatic hernia, other musculoskeletal/integumental anomalies; all chromosomal anomalies—Down syndrome, other chromosomal anomalies; all other congenital anomalies.

Table 2. Characteristics of 1995–1997 live births.

Characteristic	Low wheat	High wheat
Number of births	33,380	10,254
Sex (%)		
Male	51.6	50.7
Female	48.4	49.3
Maternal age (%)		
≥ 35	11.5	12.6
< 35	88.5	87.4
Maternal education (%)		
< High school	6.8	7.6
High school graduate	92.6	92.0
Unknown	0.6	0.4
Marital status (%)		
Unmarried	13.7	10.7
Married	86.3	89.3
Parity (%)		
1	27.5	27.1
≥ 2	72.3	72.9
Unknown	0.2	0.0
Prenatal care (%)		
None or started after first trimester	12.7	16.3
Started first trimester	84.8	83.1
Unknown	2.5	0.5
Previous preterm or SGA (%)		
Yes	2.1	1.8
No	95.9	96.9
Unknown	2.1	1.2
Tobacco use during pregnancy (%)		
Yes	11.6	9.9
No	75.3	69.6
Unknown	13.1	20.5
Alcohol use during pregnancy (%)		
Yes	0.6	0.7
No	86.0	78.7
Unknown	13.4	20.6
Season of conception (%)		
April–June (spring)	24.3	24.2
Other months	74.7	75.6
Unknown	1.0	0.2
Births with missing information (%)		
Malformation	2.1	1.2
Gestational age	1.0	0.2
SGA	1.0	0.2

Significant increases were observed for circulatory/respiratory and musculoskeletal/integumental anomalies among combined male and female births, and for infant death from congenital anomalies among boys. Among births with circulatory/respiratory anomalies [*International Classification of Diseases*, 9th revision (ICD-9 1989) 745–748], adjustment for the significant covariates (maternal age and conception during April–June, peak time of herbicide application) did not result in a change for the effect of high-wheat counties, as shown by the following adjusted ORs: combined male–female, OR = 1.64 (95% CI, 1.06–2.53); males, OR = 1.81 (95% CI, 1.05–3.11); females, OR = 1.65 (95% CI, 0.89–3.04). Further analysis of the circulatory/respiratory category showed that the strongest effects were observed for the "other" subcategory (ICD-9 747–748), which excludes heart malformations (ICD-9 745–746) but includes anomalies of the aorta, patent ductus arteriosus, other anomalies of the circulatory system, and all anomalies of the respiratory system. Among births with these "other" circulatory/respiratory malformations, the only significant covariate was conception during April–June. Adjustment for this covariate did not change the high-wheat effects presented in Table 3, as shown by the following adjusted ORs: combined male–female, OR = 1.99 (95% CI, 1.12–3.53); males, OR = 2.02 (95% CI, 1.01–4.02); females, OR = 2.06 (95% CI, 0.92–4.61). ORs for the effect of conception during April–June were as follows: combined male–female, OR = 1.75 (95% CI, 1.09–2.80); males, OR = 2.42 (95% CI, 1.42–4.15); females, OR = 1.02 (95% CI, 0.45–2.34). Given both wheat production and month of conception, boys conceived during April–June and born in high-wheat counties were almost five time more likely to be diagnosed with a birth anomaly coded as ICD-9 747–748 than were boys in low-wheat counties conceived during other months of the year. The difference of the seasonality effect between boys and girls was confirmed by a statistical test for interaction between sex and conception during April–June (p = 0.05). Low-wheat and high-wheat counties in separate analyses showed similar increases for circulatory/respiratory anomalies, excluding heart malformations. among boys conceived during April–June: low-wheat counties, OR = 2.04 (95% CI, 1.08–3.84); high-wheat counties, OR = 2.13 (95% CI, 1.26–3.64). In other words, the more than 2-fold increase for these anomalies among April–June conceptions was not specifically tied to chlorophenoxy herbicides. Other herbicide use needs to be considered, for example, dicamba.

Increases observed for musculoskeletal/integumental anomalies (ICD-9 754–757) were mostly associated with subcategories poly-/syn-/adactyly and "other musculoskeletal/integumental malformations." Adjustment for parity and prenatal care, the only maternal covariates showing a significant effect, did not affect notably the high-wheat effect, as shown by the following adjusted ORs presented for combined males and females only: all musculoskeletal/integumental anomalies, OR = 1.50 (95% CI, 1.06–2.12); poly-/syn-/adactyly, OR = 2.27 (95% CI, 1.16–4.43); other musculoskeletal/integumental anomalies, OR = 1.72 (95% CI, 1.11–2.67). Conception during April–June was not associated with an increase in musculoskeletal/integumental anomalies.

Infant death from congenital anomalies was significantly increased among boys but not among girls, with most deaths caused by heart and musculoskeletal anomalies. This difference between boys and girls was confirmed by a statistical test for interaction between the high-wheat county group and sex (p = 0.002).

The male:female ratios of births with any congenital anomaly were 1.67 and 1.60 in the low- and high-wheat counties, respectively, whereas these ratios for all births were 1.07 and 1.03 for low- and high-wheat counties, respectively, suggesting that males may be more susceptible to congenital anomalies than are girls. Similar observations about the sex ratios have been made previously (Francannet et al. 1993; Garry et al. 1996, 2002; Imaizumi et al. 1991).

Additional congenital anomalies, not diagnosed at birth, were identified from death certificates for 24 infants, including 20 infants with circulatory/respiratory anomalies. Combining these additional cases with those obtained from birth records, the OR for births with any anomaly (combined boys and girls) did not change (OR = 1.07; 95% CI, 0.87–1.30), whereas the OR for births with circulatory/respiratory anomalies decreased slightly (OR = 1.55; 95% CI, 1.04–2.32).

Discussion

Results from this study indicate that in rural, agricultural counties where wheat acreage occupies a larger percentage of the land and where use of chlorophenoxy herbicides is higher, anomalies of the circulatory/respiratory and musculoskeletal/integumental system significantly increased. To interpret these results, one should bear in mind the choice of reference group. The advantage of selecting rural, agricultural, low-wheat counties as referent was that counties included in the study would be more alike, except for wheat acreage, the factor under investigation. The disadvantage was that the reference group was not a null-data referent. If, for example, a specific anomaly had been associated with both chlorophenoxy herbicides in high-wheat counties and other herbicides applied to corn and soy beans in low-wheat counties, no effect might have been observed for this anomaly in high-wheat counties. In other words, use of low-wheat counties as the referent may have produced an underestimate of effects in association with high-wheat counties. Although selection of urban counties as referent would have had the advantage of little or no exposure to agricultural herbicides, the disadvantage would have been that other, nonagricultural factors might also be involved in causing a lower level of birth malformations in urban counties. For example, easier access to prenatal care may be associated with elective abortion after prenatal diagnosis of an anomaly (Cragan and Khoury 2000). Underreporting may be more frequent in urban than in rural counties, because in large hospitals information on birth malformations is provided by obstetricians, in contrast to small hospitals, where pediatricians are the source of information (Hexter and Harris 1991). Therefore, selection of urban counties as referent would likely have overestimated the effects in high-wheat counties. The contribution of this study is that by selecting rural, agricultural, low-wheat counties as referent, effect estimates, although conservative, could be more specifically tied to chlorophenoxy herbicides and/or contaminants.

Several limitations of this study need to be considered. Data for this study were based on birth certificates and therefore subject to significant underreporting (Snell et al. 1992). For example, only 28% of malformations recognizable at birth among 1989–1990 Georgia births, reported by the Metropolitan Atlanta Congenital Defects Program, were also reported by Georgia birth certificates (Watkins et al. 1996). In the present study, fewer than 2% of birth certificates were not marked for presence or absence of malformations, in contrast to at least 8% in urban counties. This supported the notion that reporting by small hospitals probably was more complete than reporting by larger hospitals in urban counties. Malformations not recognizable at birth, especially the occurrence of heart malformations among newborns discharged earlier from the hospital, could have contributed significantly to underreporting (Gadow et al. 1996; Watkins et al. 1996). Combining birth malformations based on organ system classification may have resulted in risk estimates of malformations that do not share the same causal agents (Kogevinas and Sala 1998). Wheat acreage per county, an indirect measure of use of chlorophenoxy herbicides, would be appropriate if the amount applied was in direct proportion to wheat acreage, which may or may not have been the case in the selected counties. Results from this ecologic study were designed to estimate regional differences. Conclusions drawn at the population level are not necessarily valid at the individual level (Morgenstern 1995).

It is remarkable that given the data limitations, the results of the present hazard-identification study are consistent with a previous birth malformation study (Garry et al. 1996). A Norwegian agricultural study reported increased rates for central nervous system anomalies, cryptorchism, hypospadias, urinary system anomalies, and limb reduction in association with grain agriculture and pesticide purchase or grain agriculture and use of spray equipment (Kristensen et al. 1997). Conceptions in spring showed an increase in birth malformations, in association with grain farming. A New Zealand study compared incidence of congenital anomalies in specific regions during years of spraying with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (1972–1976) with that in years of no spraying (1959–1965). 2,4,5-T is chemically related to 2,4-D and MCPA. Births with any malformation, heart malformations, hypospadias, or clubfoot were significantly increased in births during the years of 2,4,5-T application (Hanify et al. 1981).

Other studies have reported increased levels of birth malformations in association with less specific pesticide exposures. An increase in transposition of the great arteries was reported among newborns, especially males, whose mother had been exposed to any pesticides during the first trimester (Loffredo et al. 2001). An increased risk for nervous system malformations, oral clefts, and multiple anomalies was

Table 3. Developmental outcomes in low-wheat and high-wheat counties for 1995–1997 live births.

	Male + female			Male			Female		
Perinatal effect	N_{LW}	N_{HW}	OR (95% CI)	N_{LW}	N_{HW}	OR (95% CI)	N_{LW}	N_{HW}	OR (95% CI)
Births with any anomaly	596	213	1.07 (0.87–1.31)	373	131	1.04 (0.81–1.33)	223	82	1.14 (0.87–1.51)
Central nervous system anomalies	50	12	0.81 (0.46–1.42)	25	7	0.97 (0.45–2.07)	25	5	0.63 (0.25–1.56)
Other central nervous system anomalies	20	5	0.79 (0.30–2.11)	NA	NA	NA	NA	NA	NA
Circulatory/respiratory anomalies	74	39	1.65 (1.07–2.55)	42	24	1.83 (1.06–3.14)	32	15	1.67 (0.90–3.09)
Heart malformations	40	15	1.23 (0.70–2.17)	21	10	1.63 (0.84–3.16)	19	5	0.90 (0.32–2.49)
Other circulatory/respiratory anomalies	42	27	2.03 (1.14–3.59)	25	16	2.05 (1.02–4.09)	17	11	2.07 (0.92–4.63)
Digestive system anomalies	81	24	0.92 (0.55–1.52)	47	11	0.74 (0.37–1.48)	34	13	1.40 (0.76–2.59)
Cleft lip/palate	46	16	1.12 (0.62–2.01)	28	10	1.17 (0.55–2.47)	18	6	1.61 (0.65–3.96)
Urogenital anomalies	123	44	1.04 (0.71–1.52)	112	37	0.97 (0.65–1.44)	11	7	2.09 (0.86–5.08)
Malformed genitalia	NA	NA	NA	25	8	1.03 (0.51–2.09)	NA	NA	NA
Other urogenital anomalies	100	35	1.01 (0.65–1.55)	91	29	0.91 (0.57–1.44)	9	6	2.11 (0.76–5.87)
Musculoskeletal/integumental anomalies	142	70	1.50 (1.06–2.12)	78	37	1.45 (0.96–2.18)	64	33	1.62 (1.01–2.60)
Poly-/syn-/adactyly	19	14	2.43 (1.26–4.71)	12	7	1.88 (0.86–4.10)	7	7	3.19 (1.08–9.39)
Club foot	33	9	0.84 (0.39–1.80)	NA	NA	NA	NA	NA	NA
Other musculoskeletal/integumental anomalies	84	47	1.70 (1.10–2.62)	41	20	1.53 (0.87–2.66)	43	27	1.91 (1.12–3.24)
Chromosomal	60	17	0.93 (0.55–1.58)	36	11	1.07 (0.54–2.13)	24	6	0.73 (0.35–1.51)
Down syndrome	32	10	1.02 (0.52–2.01)	NA	NA	NA	NA	NA	NA
Other chromosomal anomalies	28	7	0.80 (0.33–1.96)	NA	NA	NA	NA	NA	NA
Other congenital anomalies	189	42	0.69 (0.49–0.98)	113	29	0.80 (0.53–1.22)	76	13	0.54 (0.27–1.08)
Gestational age < 37 weeks	2,304	748	1.05 (0.95–1.16)	1,277	416	1.08 (0.94–1.24)	1,027	332	1.02 (0.90–1.16)
Small for gestational age	1,552	503	1.05 (0.94–1.17)	836	258	1.02 (0.90–1.16)	716	245	1.09 (0.92–1.29)
Infant death from congenital anomalies	55	22	1.27 (0.80–2.00)	23	17	2.66 (1.52–4.65)	32	5	0.48 (0.20–1.15)

Abbreviations: NA, data not analyzed due to low number of observations; N_{LW}, number of births in low-wheat counties; N_{HW}, number of births in high-wheat counties; OR, unadjusted odds ratio. The following birth anomalies were included in the combined categories, based on organ system classification, but were not analyzed as single categories due to low number of observations: anencephalus, spina bifida/meningocele, hydrocephalus, microcephalus, rectal atresia/stenosis, tracheo-esophageal fistula, omphalocele, other gastrointestinal anomalies, renal agenesis, diaphragmatic hernia. The following birth totals were used in the calculation of odds ratios and confidence intervals: Birth malformations: low-wheat, 32,674 (male, 16,859; female, 15,815); high-wheat, 10,129 (male, 5,132; female, 4,997). Gestational age: low-wheat, 33,054 (male, 17,075; female, 15,979); high-wheat, 10,234 (male, 5,183; female, 5,051). SGA: low-wheat, 33,047 (male, 17,073; female, 15,974); high-wheat, 10,233 (male, 5,183; female, 5,050). Infant death: low-wheat, 33,380 (male, 17,227; female, 16,153); high-wheat, 10,254 (male, 5,194; female, 5,060).

reported among offspring of mothers involved in agricultural activities during 1 month before and 3 months after conception, with presumably low levels of pesticide exposure (García et al. 1999). An association was observed between orchidopexy rates and level of pesticide use in agricultural regions in Spain (García-Rodríguez et al. 1996).

Other abnormalities reported in association with chlorophenoxy herbicide exposure may contribute to adverse developmental or reproductive effects: for example, increased risk of abortion at less than 12 weeks of gestation for preconception exposure (Arbuckle et al. 1999a, 2001); or increased levels of asthenospermia, necrospermia, and teratospermia in farm sprayers who applied 2,4-D (Lerda and Rizzi 1991).

Toxicologic studies have reported adverse developmental outcomes in rodent models. 2,4-D is teratogenic (Schardein 1993). Pure 2,4-D has been shown to be maternally toxic, embryolethal, and a potential inducer of kidney and urogenital malformations in rats (Fofana et al. 2000). Supernumerary ribs were observed in rat litters treated with 2,4-D (Chernoff et al. 1990). A review article on 2,4-D safety concluded that reproductive and developmental effects occur in toxicologic studies, but mostly at maternally toxic doses, and that no effects were expected at the low levels humans were exposed to (Munro et al. 1992). However, recent studies indicate that chlorophenoxy herbicides at low doses do have biologic effects, although not necessarily teratogenic effects. Reduced litter size was observed in pregnant mice exposed to low and environmentally relevant doses (0.01 mg/kg/day) in their drinking water of a commercial formulation of herbicides consisting of 2,4-D, mecoprop, dicamba, and inactive ingredients (Cavieres et al. 2002). An increase of the lymphocyte replicative index was observed for *in vitro* tests at a low dose (0.005 mM) of commercial 2,4-D (Holland et al. 2002). Exposure to 2,4-D may involve endocrine disruption due to interference of 2,4-D with thyroid hormone transport carriers (Van den Berg et al. 1991).

Toxicity of chlorophenoxy herbicides is usually tested on pure or reagent-grade compounds. Biologic responses to these herbicides in presence of contaminants, adjuvants, and fertilizer may be higher. Contaminants present in technical grade 2,4-D depend on the purity of the chemicals used to produce 2,4-D, and on the production process (IARC 1986). Occasionally 2,3,7,8-tetrachlorodibenzo-*p*-dioxin may be present in technical grade 2,4-D (Johnson et al. 1992). Adjuvants may contribute to adverse health effects (Garry et al. 1999). *In vitro* assays showed that commercial grade 2,4-D at concentrations of 1 and 10 µg/mL caused cell proliferation, whereas assays with reagent grade 2,4-D did not. In a review on health effects of chlorophenoxy herbicides, Sterling and Arundel (1986) suggested that not only the contaminant dioxins and furans may have carcinogenic and teratogenic properties, but also the uncontaminated phenoxy herbicides themselves. Two toxicologic studies listed above seem to support this notion: Fofana et al. (2000) used pure 2,4-D, and Van den Berg et al. (1991) used chemicals "of the highest purity commercially available." It is not known whether the chlorophenoxy herbicides themselves, their contaminants and adjuvants, or a combination of these chemicals with other agents are involved with the observed excess of birth malformations.

To deal with the complexity of the current chemicalization of our environment, several investigators have suggested that individual risk-factor epidemiology may not be ideal because many of the exposures may be common throughout the study population in a given geographic region (Kogevinas and Sala 1998; McMichael 2002; Pekkanen and Pearce 2001; Shy 1997; Susser 1998). They suggested further that population studies are fundamental in identifying public health problems and that interregion comparison may offer comparison data for the population at risk. Enhanced sensitivity and resolution of environmental toxicant effects within one region can then be garnered by use of more sophisticated molecular toxicant studies in the affected population (Pekkanen and Pearce 2001).

In conclusion, although results from the present study should be viewed with caution, the consistency of these results with other studies points to a potentially hazardous scenario in terms of specific excess birth defects. Future, more targeted studies investigating developmental effects from chlorophenoxy herbicides and/or contaminants should focus on the toxicology of these chemicals at levels as they occur in the environment and on the routes of exposure for pregnant women living in high-wheat regions.

REFERENCES

Arbuckle TE, Lin Z, Mery LS. 2001. An exploratory analysis of the effect of pesticide exposure in the risk of spontaneous abortion in an Ontario farm population. Environ Health Perspect 109:851–857.

Arbuckle TE, Savitz DA, Mery LS, Curtis KM. 1999a. Exposure to phenoxyherbicides and the risk of spontaneous abortion. Epidemiology 10:752–760.

Arbuckle TE, Schrader SM, Cole D, Hall JC, Bancej CM, Turner LA, et al. 1999b. 2,4-Dichlorophenoxyacetic acid residues in semen of Ontario farmers. Reprod Toxicol 13:421–429.

Axelson O, Sundell L. 1974. Herbicide exposure, mortality and and tumor incidence. An epidemiologic investigation on Swedish railroad workers. Work Environ Health 11:21–28.

Axelson O, Sundell L, Andersson K, Edling C, Hogstedt C, Kling H. 1980. Herbicide exposure and tumor mortality. An updated epidemiologic investigation on Swedish railroad workers. Scand J Work Environ Health 6:73–79.

Cavieres MF, Jaeger J, Porter W. 2002. Developmental toxicity of a commercial herbicide mixture in mice. I. Effects on embryo implantation and litter size. Environ Health Perspect 110:1081–1085.

Chernoff N, Setzer RW, Miller DB, Rosen MB, Rogers JM. 1990. Effects of chemically induced maternal toxicity on prenatal development in the rat. Teratology 42:651–658.

Cragan JD, Khoury MJ. 2000. Effect of prenatal diagnosis on epidemiologic studies of birth defects. Epidemiology 11:695–699.

Curl CL, Fenske RA, Kissel JC, Shirai JH, Moate TF, Griffith W, et al. 2002. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. Environ Health Perspect 110:A787–792.

Eriksson M, Hardell L, Berg NO, Möller T, Axelson O. 1981. Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. Br J Ind Med 38:27–33.

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. 1996. Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations. Occup Environ Med 53:583–585.

Fofana D, Kobae H, Oku S, Nishi J, Miyata K. 2000. Prenatal developmental effects of pure 2,4-dichlorophenoxyacetic acid (2,4-D) on the rat. Congenit Anom 40:287–296.

Francannet C, Lancaster PA, Pradat P, Cocchi G, Stoll C. 1993. The epidemiology of three serious cardiac defects. A joint study between five centres. Eur J Epidemiol 9:607–616.

Gadow EC, Otaño L, Lippold SE. 1996. Congenital malformations. Curr Opin Obstet Gynecol 8:412–416.

García AM, Fletcher T, Benavides FG, Orts E. 1999. Parental agricultural work and selected congenital malformations. Am J Epidemiol 149:64–74.

García-Rodríguez J, García-Martín M, Nogueras-Ocaña M, de Dios Luna-del-Castillo J, Espigares García M, Olea N, et al. 1996. Exposure to pesticides and cryptorchidism: geographical evidence of a possible association. Environ Health Perspect 104:1090–1095.

Garry VF, Burroughs B, Tarone R, Kesner JS. 1999. Herbicides and adjuvants: an evolving view. Toxicol Ind Health 15:159–167.

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. Environ Health Perspect 110(suppl 3):441–449.

Garry VF, Schreinemachers D, Harkins ME, Griffith J. 1996. Pesticide appliers, biocides, and birth defects in rural Minnesota. Environ Health Perspect 104:394–399.

Hanify JA, Metcalf P, Nobbs CL, Worsley KJ. 1981. Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science 212:349–351.

Hardell L. 1981. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand J Work Environ Health 7:119–130.

Hardell L, Eriksson M. 1988. The association between soft tissue sarcomas and exposure to phenoxyacetic acids. A new case-referent study. Cancer 62:652–656.

Hardell L, Eriksson M, Lenner P, Lundgren E. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 43:169–176.

Hardell L, Sandström A. 1979. Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br J Cancer 39:711–717.

Harris SA, Solomon KR. 1992. Human exposure to 2,4-D following controlled activities on recently sprayed turf. J Environ Sci Health B27(1):9–22.

Hexter AC, Harris JA. 1991. Bias in congenital malformations information from the birth certificate. Teratology 44:177–180.

Hill RH, To T, Holler JS, Fast DM, Smith SJ, Needham LL, et al. 1989. Residues of chlorinated phenols and phenoxy acid herbicides in the urine of Arkansas children. Arch Environ Contam Toxicol 18:469–474.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, et al. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 256:1141–1147.

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, et al. 2002. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid *in vitro* and *in vivo*. Mutat Res 521:165–178.

IARC. 1986. Some halogenated hydrocarbons and pesticide exposures. IARC Monogr Eval Carcinog Risk Chem Hum 41:357–406.

Imaizumi Y, Yamamura H, Nishikawa M, Matsuoka M, Moriyama I. 1991. The prevalence at birth of congenital malformations at a maternity hospital in Osaka City, 1948–1990. Jpn J Hum Genet 36:275–287.

Johnson ES, Parsons W, Weinberg CR, Shore DL, Mathews J, Patterson DG, et al. 1992. Current serum levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in phenoxy acid herbicide applicators and characterization of historical levels. J Natl Cancer Inst 84:1648–1653.

Kogevinas M, Sala M. 1998. Pesticides and congenital malformations—how many studies will it take to reach a conclusion? Scand J Work Environ Health 24:445–447.

Kristensen P, Irgens LM, Andersen A, Bye AS, Sundheim L. 1997. Birth defects among offspring of Norwegian farmers, 1967–1991. Epidemiology 8:537–544.

Larney FJ, Cessna AJ, Bullock MS. 1999. Herbicide transport on wind-eroded sediment. J Environ Qual 28:1412–1421.

Lerda D, Rizzi R. 1991. Study of reproductive function in persons occupationally exposed to 2,4-dichlorophenoxyacetic acid (2,4-D). Mutat Res 262:47–50.

Loffredo CA, Silbergeld EK, Ferencz C, Zhang J. 2001. Association of transposition of the great arteries in infants with maternal exposures to herbicides and rodenticides. Am J Epidemiol 153:529–536.

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, et al. 2002. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomark Prev 10:1155–1163.

McMichael AJ. 2002. Population, environment, disease, and survival: past patterns, uncertain futures. Lancet 359:1145–1148.

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

Morrison H, Savitz D, Semenciw R, Hulka B, Mao Y, Morison D, et al. 1993. Farming and prostate cancer mortality. Am J Epidemiol 137:270–280.

Munro IC, Carlo GL, Orr JC, Sund KG, Wilson RM, Kennepohl E, et al. 1992. A comprehensive, integrated review and evaluation of the scientific evidence relating to the safety of the herbicide 2,4-D. J Am Coll Toxicol 11:559–664.

NCHS. 1995. Linked Birth/Infant Death Birth Cohort File. Hyattsville, MD:National Center for Health Statistics.

———.1996. Linked Birth/Infant Death Birth Cohort File. Hyattsville, MD:National Center for Health Statistics.

———. 1997. Linked Birth/Infant Death Birth Cohort File. Hyattsville, MD:National Center for Health Statistics.

———. 1998. Vital Statistics of the United States. Natality. Hyattsville, MD:National Center for Health Statistics. Available: http://www.cdc.gov/nchs/data/lbid/techap98.pdf [accessed 9 May 2003].

Nishioka MG, Burkholder HM, Brinkman MC, Gordon SM, Lewis RG. 1996. Measuring transport of lawn-applied herbicide acids from turf to home: correlation of dislodgeable 2,4-D turf residues with carpet dust and carpet surface residues. Environ Sci Technol 30:3313–3320.

Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR. 2001. Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. Environ Health Perspect 109:1185–1191.

Pekkanen J, Pearce N. 2001. Environmental epidemiology: challenges and opportunities. Environ Health Perspect 109:1–5.

Persson B, Dahlander AM, Fredriksson M, Noorlind Brage H, Ohlson CG, Axelson O. 1989. Malignant lymphomas and occupational exposures. Br J Ind Med 46:516–520.

Renne DS, Wolf MA. 1979. Experimental studies of 2,4-D herbicide drift characteristics. Agric Meteorol 20:7–24.

SAS Institute. 2001. SAS/STAT Guide for Personal Computers, Version 8. Cary, NC:SAS Institute, Inc.

Schardein JL. 1993. Chemically Induced Birth Defects. 2nd ed. New York:Marcel Dekker, Inc.

Schreinemachers DM. 2000. Cancer mortality in four northern wheat-producing states. Environ Health Perspect 108:873–881.

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

Shy CM. 1997. The failure of academic epidemiology: witness for the prosecution. Am J Epidemiol 145:479–484.

Snell LM, Little BB, Knoll KA, Johnston WL, Rosenfeld CR, Gant NF. 1992. Reliability of birth certificate reporting of congenital anomalies. Am J Perinatol 9:219–222.

Sterling TD, Arundel AV. 1986. Health effects of phenoxy herbicides. A review. Scand J Work Environ Health 12:161–173.

Susser M. 1998. Does risk factor epidemiology put epidemiology at risk? Peering into the future. J Epidemiol Community Health 52:608–611.

USDA. 1992a. Agricultural Census. Washington, DC:U.S. Department of Agriculture, National Agricultural Statistics Service. Available: http://www.usda.gov/nass [accessed 15 March 2002].

———. 1992b. Agricultural Chemical Usage, Field Crop Summary 1992. Washington, DC:U.S. Department of Agriculture. Available: http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/ [accessed 15 March 2002].

USGS. 1997. Pesticides in the Atmosphere. Factsheet FS-152-95. Sacramento, CA:U.S. Geological Survey. Available: http://ca.water.usgs.gov/pnsp/atmos [accessed 15 March 2002].

———. 2001. The Quality of Our Nation's Waters. Nutrients and Pesticides. Circular 1225. Washington, DC:U.S. Geological Survey. Available: http://water.usgs.gov/pubs/circ/circ1225 [accessed 25 February 2003].

Van den Berg KJ, van Raaij JAGM, Bragt PC, Notten WRF. 1991. Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels *in vivo*. Arch Toxicol 65:15–19.

Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A, et al. 1986. Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. Scand J Work Environ Health 13:9–17.

Waite DT, Cessna AJ, Grover R, Kerr LA, Snihura AD. 2002. Environmental concentrations of agricultural herbicides: 2,4-D and triallate. J Environ Qual 31:129–144.

Watkins ML, Edmonds L, McClearn A, Mullins L, Mulinare J, Khoury M. The surveillance of birth defects: the usefulness of the revised US standard birth certificate. 1996. Am J Public Health 86:731–734.

Wigle DT, Semenciw RM, Wilkins K, Riedel D, Ritter L, Morrison HI, et al. 1990. Mortality study of Canadian male farm operators: non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. J Natl Cancer Inst 82:575–582.

Williams RL, Creasy RK, Cunningham GC, Hawes WE, Norris FD, Tashiro M. 1982. Fetal growth and perinatal viability in California. Obstet Gynecol 59:624–632.

Wingren G, Fredrikson M, Noorlind Brage H, Nordenskjöld B, Axelson O. 1990. Soft tissue sarcoma and occupational exposures. Cancer 66:806–811.

Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG. 1987. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in western Washington. J Natl Cancer Inst 78:899–910.

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, et al. 1990. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1:349–356.