Birth Defects, Season of Conception, and Sex of Children Born to Pesticide Applicators Living in the Red River Valley of Minnesota, USA

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We previously demonstrated that the frequency of birth defects among children of residents of the Red River Valley (RRV), Minnesota, USA, was significantly higher than in other major agricultural regions of the state during the years 1989–1991, with children born to male pesticide applicators having the highest risk. The present, smaller cross-sectional study of 695 families and 1,532 children, conducted during 1997–1998, provides a more detailed examination of reproductive health outcomes in farm families ascertained from parent-reported birth defects. In the present study, in the first year of life, the birth defect rate was 31.3 births per 1,000, with 83% of the total reported birth defects confirmed by medical records. Inclusion of children identified with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Conceptions in spring resulted in significantly more children with birth defects than found in any other season (7.6 vs. 3.7%). Twelve families had more than one child with a birth defect (n = 28 children). Forty-two percent of the children from families with recurrent birth defects were conceived in spring, a significantly higher rate than that for any other season. Three families in the kinships defined contributed a first-degree relative other than a sibling with the same or similar birth defect, consistent with a Mendelian inheritance pattern. The remaining nine families did not follow a Mendelian inheritance pattern. The sex ratio of children with birth defects born to applicator families shows a male predominance (1.75 to 1) across specific pesticide class use and exposure categories exclusive of fungicides. In the fungicide exposure category, normal female births significantly exceed male births (1.25 to 1). Similarly, the proportion of male to female children with birth defects is significantly lower (0.57 to 1; p = 0.02). Adverse neurologic and neurobehavioral developmental effects clustered among the children born to applicators of the fumigant phosphine (odds ratio [OR] = 2.48; confidence interval [CI], 1.2–5.1). Use of the herbicide glyphosate yielded an OR of 3.6 (CI, 1.3–9.6) in the neurobehavioral category. Finally, these studies point out that *a*) herbicides applied in the spring may be a factor in the birth defects observed and *b*) fungicides can be a significant factor in the determination of sex of the children of the families of the RRV. Thus, two distinct classes of pesticides seem to have adverse effects on different reproductive outcomes. Biologically based confirmatory studies are needed. *Key words:* birth defects, pedigree, pesticides, season, sex ratio. *Environ Health Perspect* 110(suppl 3):441–449 (2002).
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Reproductive health concerns raised in our earlier studies of genotoxicity in pesticide applicators led us to speculate that the genomic instability we observed in exposed workers and expressed as increased chromosome rearrangements in G-banded lymphocytes and/or molecular rearrangements of the human T-cell V(d)J region might also result in developmental and other reproductive toxicity including birth defects (*1,2*). As a first effort, we examined the frequencies of birth defects by crop-growing region in Minnesota (*3*) during 1989–1991. The Red River Valley (RRV) region (northwestern Minnesota, USA) showed the highest age-adjusted rate of birth defects compared with urban areas of Minnesota (26.9 vs. 18.5/1,000 live births). The RRV is a major wheat, sugar beet, and potato crop–growing region of our state. At times, these crops require extensive use of fungicides and routinely require large amounts of chlorophenoxy herbicides. Herbicides are applied in the spring, insecticides in the summer, and fungicides as needed in the summer and fall. Fumigants are routinely applied to stored harvested grain in the fall. Pesticide applicators from the RRV region demonstrated even higher age-adjusted levels of birth defects (30.0/1,000 live births) than did the general population from the region. The ratio of male to female children with birth defects was significantly increased in families of pesticide applicators from the RRV. The method of birth defect ascertainment employed in the initial study was birth records provided to the Minnesota Department of Health by local health care providers at the time of birth. Because of the limited reporting time frame (birth to hospital discharge of the newborn), the initial study results were subject to substantial underascertainment. The present cross-sectional study was undertaken to provide more detailed information regarding the reproductive health of pesticide applicators and their families.

Materials and Methods

Overview

Population and population access. In Minnesota, licensing for application of pesticides commercially or for application to one's own farmland requires periodic recertification by completion of a program of education and examination. Applicators are licensed to apply specific classes of pesticides (herbicides, insecticides, fungicides, and/or fumigants). Through the Minnesota Department of Agriculture and the Minnesota Extension Service, we were provided with an updated list of licensees covering the time frame from 1991 through 1996.

Survey strategy. In the five-county RRV study area, 3,000 residents were licensed during the time frame cited. Fifteen hundred persons were randomly selected for our umbrella study of general health and pesticide use. The location of 1,340 licensees was confirmed through telephone and postcard follow-up. One thousand seventy (1,070) applicators (98% male) volunteered to participate by phone interview for the general health and pesticide use study. Of this group, 851 were married or had a marriage-like relationship. Eight hundred two partners (women) participated in the general health and pesticide use survey. Each partner was interviewed by phone at a time separate from interview of the applicator.

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Reproductive health assessment. During the phone interview, applicators and/or their partners were also invited to participate and return a detailed written reproductive health and pesticide use assessment questionnaire.

Informants for the reproductive study were 228 male spouse only (pesticide applicator), 90 female spouse only, and 377 couples. Thus, at least one member of 695 families responded and gave detailed information regarding reproductive health and pesticide use. Of the 695 families, self-reports from 133 informant families indicated that they did not have children. Of this group, 54 stated they did not want to have children, 15 stated difficulty having children, and 64 gave no detailed statement regarding child-bearing status. Each written survey was sent separately to the applicators and their partners. In toto, this stepped approach to detailed reproductive health survey met University of Minnesota institutional review board approval and concerns regarding privacy and data reporting.

Eligibility Criteria

General. All self-reported live births fathered by a pesticide applicator (n = 536) were considered in this study. Live births and children with birth defects were defined by written survey. Findings regarding pregnancy losses, including miscarriages, are discussed elsewhere (*4*). In the present effort, birth defects occurring in a total of 1,532 live births were considered in these analyses.

Births and congenital anomalies. Each study participant was provided with a written consent form to allow us to follow-up and confirm parent-reported reproductive health information through birth certificate and medical records examination. For subjects with adult children (>18 years of age), specific written consent by the adult child was required for these confirmatory studies. Where specific permission was given by a parent or adult child, follow-up on anatomic (structural) anomalies was performed. Pertinent medical records were obtained from the cooperating clinic or medical facility of record through the allied health staff and/or medical records department. Each clinical note and diagnosis was reviewed by a physician and genetic research associate. Our assessments also included clinically diagnosed and/or laboratory test–defined genetic and metabolic anomalies that have an anatomic component (e.g., Marfan's syndrome, gangliosidosis, Poland's syndrome, and Down's syndrome). All reported anomalies were recorded according to the *International Classification of Disease, 9th Revision* (*5*). Birth anomalies were grouped according to major organ system as previously described (*3*). Confounding variables such as maternal smoking, drinking, age, and chronic diseases such as diabetes and hypertension were examined. In this retrospective study, where possible, familial genetic history (pedigree), pregnancy medication use, and nonmedicinal drug use (including vitamins) were assessed in families with birth defects. Developmental neurobehavorial conditions such as autism, attention deficit disorder (ADD), and its variants were not considered in our detailed follow-up studies because of limited access to uniform diagnostic neurobehavioral information.

Families Reporting Multiple Children with Birth Anomalies

Detailed genetic and familial histories were obtained through follow-up questionnaire and brief interview of these study subject families. Consanguinity, occurrence of birth anomalies, and similar birth anomalies among parents, their siblings, and the children of their siblings were considered. Where possible, a pedigree was constructed detailing birth anomalies, cause of death, and age at death of family members, including grandparents and their siblings.

Pesticide Use

Each certified pesticide applicator was initially interviewed by phone regarding current and past pesticide use in agriculture with specific attention to product name, years used, and the number of days per year applied. Approximately 6 months later, where possible, the subject was reinterviewed by written questionnaire to document common pesticide use by pesticide class, acreage treated, type of crop, and use of personal protective gear. Overlap between the two questionnaires was intentional to validate use of pesticides by class (herbicides, insecticides, fumigants, fungicides). Phone survey pesticide use information was employed in our assessments in those instances where follow-up written pesticide use information was lacking. Similarly, the spouse of the applicator was interviewed by phone and again by written questionnaire. Involvement in the processes associated with pesticide use, both historically and currently, was assessed. Personal use of pesticides by spouses was also ascertained. Where possible, specific pesticide exposure and/or use was identified.

Statistics

Where appropriate, regression analysis, two-sided t-tests, and analysis of variance methods were employed. Variables considered for regression analysis included mother's age, smoking status, alcohol use, and season of conception. Chronic diseases such as diabetes, pharmacologically treated hypertension, and arthritis and occupations other than agriculture were considered separately. Specific medication use during pregnancy and dietary information were not considered in our survey. Residence at a rural site (towns with populations <3,000) or on a farm during childhood (<18 years of age) was considered a factor in some of these statistical analyses.

Results

General

Seventy children born to 536 couples had one or more birth defects (Table 1). Five children had other developmental anomalies: autism (n = 2) and diabetes (n = 3) diagnosed at age 3 or older. Detailed confirmatory studies were performed through medical records and birth certificate examination. Medical records and/or birth certificate confirmation were obtained for 54 of 70 children (77.1%) with birth defects. Other parent-reported children with birth defects where the male spouse was not the father (n = 3) and/or was not an applicator (n = 3) were excluded from analyses. Similarly, of 19 children with ADD/attention-deficit hyperactivity disorder (ADHD), 5 were excluded from data analyses because the male applicator spouse was reported as not being the biologic father.

The mean age (±SD) of the parents at the time of birth of each child (mother, 25.4 ± 4.8 years; father, 28.5 ± 5.3) with a birth defect and those families with children without birth defects (mother, 26.4 ± 4.9 years; father, 28.7 ± 5.3) were not significantly different. The survey-reported frequencies of smokers, ex-smokers, and nonsmokers are not different among male or female parents of children with or without birth defects. The frequency of drinking alcoholic beverages (weekly, monthly) among the parents of children with and without birth defects was no different. Birth years covered in this study, by quartiles, range from 1968 to 1998, with the median frequency of all births being in 1978.

Table 1. Births, birth defects, and other developmental abnormalities.[a]

Population	Totals
Live births[b]	1,532
Family participants	695
Families with children	536
Congenital birth anomalies	70
Autism	2
Childhood diabetes	3
ADD/ADHD	14

[a]The table lists all births and birth and developmental anomalies reported by study subjects where the pesticide applicator is the father. [b]Includes twins and other multiple births.

Comparison of Birth Defect Frequencies Identified in the First Year of Life and Later

The frequency of birth defects during the first year of life reported for this cross-sectional study shows a 1.5-fold or more increase in the frequency of most birth defects compared with the birth certificate data from our earlier cohort study (Table 2). The overall frequency of birth defects in the categories examined is significantly higher in the cross-sectional study compared with the cohort study when all parent-reported birth defects are considered (p = 0.005) but not when only confirmed cases are considered (p = 0.10). Curiously, our birth record–reported frequencies for 1989–1991 (*3*) for urogenital birth defects are nearly identical to those found in the present study covering birth years from 1968 to 1998. These data suggest that the rate of urogenital birth defects is constant over time and that these anatomic defects are, for the most part, observable at birth. Confirmation by birth or death records from our cross-sectional study accounted for only 60% of the total birth defects confirmed through medical records assessment of the first year of life.

Fifty-eight of 76 birth defects recorded (Table 3) were confirmed by medical records review (76.3%). In 15 cases (19.7%), access to medical records was denied by an adult child. The remaining birth defects (4%) remained unconfirmed but were accounted for either by hospital closure and/or with no record found by the medical reporting facility. As indicated in Table 3, 70 children had birth defects; 6 of these children had more than one birth defect (7.0%) for a total of 76 birth defects. We elected to include all parent-reported birth defects in our analyses based on the data reported above.

Table 2. Comparison of the frequency of self-reported (cross-sectional study) birth anomalies with birth certificate–based cohort study.[a]

	Number of cases (n) and rate (R) of birth defects per 1,000 live births recorded in the first year of life				
	Cross-sectional study			Cohort study (1989–1992)	
Organ system	n (self-reported)	n (confirmed)	R[b]	n	R
Central nervous system	7[c]	7	4.6	6	1.3
Cardiovascular/respiratory	8	8	5.2	17	3.7
Musculoskeletal/integument	21	15	9.8	30	6.5
Gastrointestinal	3	3	2.0	6	1.3
Urogenital	6	6	4.0	20	4.4
Chromosome, genetic, metabolic	3	1	0.65	8	1.7
Totals	48	40	26.1	87	18.9

[a]In these crude comparisons, the overall frequency of self-reported and medically confirmed birth anomalies recorded in the first year of life is higher than those recorded in our earlier birth certificate–based cohort study (*3*). Regardless of ascertainment method, urogenital birth defects were reported at about the same rate (4.0 vs. 4.4 per 1,000). [b]Birth defect rate in cases confirmed by medical records within the first year of life. [c]Includes anomalies of the eye and hearing.

Table 3. Birth defects, major organ system, parent report confirmation, and age at diagnosis.[a]

		Age at reported diagnosis			Confirmation by medical records	Refusal by adult child
Birth defect	No. cases	<1 years	1–3 years	>3 years		
Central nervous system	14	7	3	4	11	3
Cardiovascular system	13	8	0	5	10	3
Gastrointestinal system	4	3	0	1	4	0
Urogenital system	9	6	1	2	8	1
Musculoskeletal system	27	17	4	6	17	7
Skin/integument	4	4	0	0	4	0
Cytogenetic/metabolic	5	3	0	2	4	1
Totals	76[b]	48	8	20	58	15

[a]Identified in the table are the number of parent-reported birth defects by organ system age at diagnosis and through medical record confirmation. More than 75% of the reported birth defects were confirmed by medical records. Approximately 80% of the adult children in the remaining 25% of cases reported refused permission for examination of medical records. [b]Five children had more than one birth defect and more than one organ system was involved. One twin birth yielded an additional case.

Recurrent Birth Defects

Pedigree analysis. Twelve families had a total of 45 children. Two or more children from each of these families had a birth defect, for a total of 28 children with one or more birth anomalies (Table 4). Other than a child's sibling, only three families (No. 1, 4, 5) in the kinships defined contributed a first-degree relative with the same or similar birth defect involving the same organ system, suggesting a familial or Mendelian origin.

Siblings from 5 of the 12 families (No. 2, 6, 8, 11, 12) with recurrent birth defects had the same or similar birth defect. These findings are consistent with larger studies that demonstrate that families with one child with a birth defect are more likely to have a second child with the same or similar birth defect (*6–8*). These data infer familial susceptibility. Two children from family No. 6 had an annular pancreas consistent with a dominant mutational event in the current generation. Case reports support this contention (*9,10*). Six families in the kinships listed (No. 2, 3, 6, 8–10) gave no prior family history of children with similar or the same birth anomalies, suggesting a nonfamilial etiology.

Four families in the group of 12 families (No. 3, 7, 9, 10) had children with different birth defects. Eight of nine children with these birth defects were conceived in spring or early summer (before 15 July). One child with a musculoskeletal deformity (from family No. 10) was conceived in fall. Previous studies by other investigators (*6–8*) conclude that families with children with one class of birth defects have a low likelihood of having a second child with a different class of birth defect. Since the birth and developmental anomalies within each of these four families differ by organ system, these untoward reproductive events suggest nonmutagenic teratogenic effects.

To summarize, the analysis of the pedigree data (Table 4) showed that 3 of the 12 families with more than one child with a birth defect follow a Mendelian or familial birth anomaly pattern. The remaining 9 families do not follow a familial pattern, at least in the limited size of the kindreds examined.

Preliminary statistical analysis. To measure the likelihood of having more than one child with a birth defect, the data were analyzed conditional on the number of children. For parents with a single child, the birth defect rate was 8.4%. For families with two children, the birth defect rate was 5.9%. One of these families had birth defects in both children, compared with an expected number of 0.3. Thus, the family is probably unusual. For families with three children, the birth defect rate was 5.7%. Six of those families had two or more children with birth defects, compared with an expected number of 1.5. Thus, four or five of the six families with multiple birth defects are unusual, whereas one or two are a chance occurrence. In families with four children, the rate was 3.9%. Two of those families had two or more children with birth defects, compared with the expected number of 0.7. Thus, one of the two families is likely unusual. In families with five children, the rate was 6.0%. Two of those families had three or more children with birth defects, compared with the expected number of 0.06. In families

Table 4. Families with more than one child with a birth defect.[a]

Subject family	Family pattern: No. of children with BD	Family pattern: Total no. of children	BD category	Multiple birth defects: 1st degree Same/similar BD	1st degree Other BD	2nd degree Same/similar BD	2nd degree Other BD
1	2	7	Cardiovascular	Yes	Yes	Yes	No
2	3	3	Musculoskeletal	No	No	No	No
3	2	3	Nervous system Musculoskeletal	No	No	No	No
4	2	3	Musculoskeletal	Yes	No	Yes	No
5	2	2	Musculoskeletal	Yes	Yes	No	No
6	2	4	Gastrointestinal	No	No	No	No
7	3	4	Skin/integumental, urogenital	No	No	Yes	Yes
8	2	3	Musculoskeletal	No	Yes	No	No
9	2	3	Musculoskeletal, nervous system	No	No	No	Yes
10	3	5	Musculoskeletal, nervous system	No	Yes	No	No
11	2	3	Musculoskeletal	No	No	Yes	Yes
12	3	5	Nervous system	No	No	Yes	Yes
Totals	28	45		3/12	4/12	5/12	4/12

BD, birth defects. [a]Reported in the table are the number of families with more than one child with a birth defect. Birth defects by major organ system, number of children with a birth defect, and the total number of children in the family are identified. First-degree relatives of the parent informant or spouse of the informant are listed in separate columns. In this scenario first-degree relatives include mother, father, brother, or sister of the informant or spouse. Children of the informant family are excluded. Second-degree relatives of parent informants (grandparents, uncles and aunts and children of siblings) are listed under a separate column. Twelve families had 28 children with a birth defect. Sixty-two percent of the total number of children born to these families ($n = 45$) had a birth defect.

with six or more children, the rate was 3.0%. One of those families with two children with birth defects cannot be considered unusual.

In sum, on the basis of this methodology, there are probably 8 or 9 unusual families among the 12 families with multiple children with birth defects. These preliminary statistical findings are reported independently of the pedigree analysis; each indicates that 9 of the 12 families studied are "unusual" or non-Mendelian and may be at special risk.

Other known contributors to special risk for recurrent birth defects are recurrent miscarriages and other fetal losses (*11*). On the basis of parent-reported data, there were no recurrent pregnancy losses (data not shown), including miscarriages and other fetal losses in the families with recurrent birth defects.

Pesticide Use, Children with Birth Defects, and Adverse Developmental Effects

Table 5 compares the relative frequency of use (%) of commonly applied pesticides reported from phone survey and in the subsequent, more detailed, self-reported written survey performed at least 6 months later. The overall results obtained from the different survey methods and instruments employed are nearly identical. These data demonstrate little difference in recall among study subjects regarding specific pesticide class use. Further, the frequency distribution of products used by applicators participating in the reproductive study was no different from that of the products applied by the general study population. Based on application practice, applicator exposure can be assessed according to specific pesticide class use groups (herbicide only; herbicide and insecticide; herbicide, insecticide, fungicide; herbicide, insecticide, fumigant; and use of all four classes of pesticides), with use of herbicides only being the referent group (*12,13*). The mean number of children per applicator per pesticide exposure group varied from 2.5 (other) to 3.0 (herbicide only) children, with a mean of 2.85 children per applicator for the entire study group. There were no significant differences in birth rate among exposure groups.

Table 5. Rank order comparison of reported use of pesticides by applicator participants from phone and written survey.[a]

Class of pesticide	No. phone respondents ($n = 1,071$)	Percent use reported	No. written survey respondents ($n = 596$)	Percent use reported
Herbicides				
Chlorophenoxy	786	73.4	385	64.6
Oxyphenoxy	380	35.5	176	29.5
Sulfonylurea	330	30.8	174	29.2
Carbanilate	303	28.3	133	22.3
Bromophenol	293	27.4	114	19.1
Benzothiazole	233	21.8	105	17.6
Nitroaniline	209	19.5	92	15.4
Insecticides				
Organophosphate	478	44.6	252	42.3
Synthetic pyrethroids	201	18.8	116	19.5
Carbamate	128	12.0	42	7.1
Fungicides				
Organotin	216	20.2	117	19.6
Ethylene bisdithiocarbamate	154	14.4	77	12.9
Triazole	129	12.0	61	10.2
Fumigants				
Phosphide	138	12.9	101	17.0
Other	4	0.3	5	0.8

[a]Listed in the table by chemical class are commonly used pesticides, including herbicides, that were applied by applicators. Five hundred ninety-six men who responded to the phone survey ($n = 1,071$) detailing general health and pesticide use, and who were married or had a marriagelike relationship ($n = 851$) participated in the written survey 6 months later detailing reproductive health and pesticide use. Comparison of the data by rank order use demonstrates that pesticide use by the applicators participating in the reproductive study was not significantly different from that of the more general RRV population participants.

Examination of the frequency of applicator families with birth defects by pesticide use class category (Table 6) shows that 15.4% of applicators who applied fumigants, insecticides, and herbicides had at least one child with a birth defect compared with 6.8% in the referent exposure group who applied only herbicides. To minimize possible bias introduced by differences in the reproductive rate per family (Table 4), odds ratio (OR) comparison of the number of applicators by pesticide use

group with and without children with birth defects was performed (Table 6). For example, 6 of 39 applicators who applied fumigants had a child with a birth defect, compared with 8 of 118 who applied herbicides only, which gave an OR = 2.27 (95% confidence interval [CI], 0.85–6.08). Comparison of birth defects to total births gave an OR of 2.0 (95% CI, 0.85–4.62) for paternal application of fumigants, insecticides, and herbicides compared with the referent group, which was not much different, indicating that the number of children per family was not a major factor in these results. Since phosphine-generating phosphides (Table 5) were the only fumigants in common use, and exposure group comparisons were suggestive, we examined in greater detail the adverse birth and developmental health effects in children born to applicators who use these phosphine-generating products. Results recorded from these more detailed efforts showed that 4 of 14 children whose father applied phosphine had birth defects involving the central nervous system. Two of 2 children with autism and 5 of 14 who had parent-reported ADD/ADHD had a biologic father who applied phosphine. Curiously, 2 of the 4 children with a central nervous system birth defect had a unilateral congenital cataract involving the right eye and no other accompanying birth defect. These children, unexpectedly, were female. Altogether, 3.8% of children whose parent used phosphine versus 1.5% of those who did not use the fumigant had adverse central nervous system or neurobehavioral sequelae (OR = 2.5; CI, 1.22–5.05). Similarly, use of the phosphonamino herbicides (glyphosate, Roundup) was overrepresented in the adverse birth and developmental effect group. Forty-three percent of the children (6 of 14) who had parent-reported ADD/ADHD used phosphonamino herbicides (OR = 3.6; CI, 1.35–9.65). No other commonly used pesticide compared by major organ and/or functional system was uniquely associated with specific adverse birth or developmental effects. Use of different classes of pesticides over the 4–6 months of agricultural pesticide use compared with the use of herbicides and no other pesticide class (herbicide use period, ~15 April to 1 July) suggests that interaction among pesticide classes used may be a factor in the birth defects observed (Table 6).

Sex of Children, Birth Defects, and Fungicide Use

Table 7 reports and compares the frequencies of birth defects among live-born male and female children according to paternal fungicide use. More male children are born to families whose male partner did not apply fungicides than to those who do apply these products ($p = 0.04$). Regarding birth defects, as expected, more male children are born with birth defects than are female children (M/F sex ratio = 1.8) when no fungicides are applied. If fungicides are applied by the male partner, far fewer male children with birth defects are born (M/F sex ratio = 0.57, $p = 0.02$). The birth rate among families of fungicide applicators with children is no different from those who apply herbicides and other products (2.85 vs. 2.80 children per family), suggesting that fertility is not a factor in these results.

Season of Conception

Conceptions in the fall led to the highest number of births but not birth defects (Table 8). Notably, conceptions in the spring led to a significantly increased number of birth defects ($p = 0.02$). Prematurity was not a factor in these results (*4*). Coincidentally, herbicides are routinely applied during the same time frame (spring). Chlorophenoxy herbicides were by far the most commonly used herbicide group (Table 5).

Discussion

General

About 3.7% of children born on an average day in the United States are said to have a birth defect (*14*). Fifty-four of 536 pesticide applicators who had children and participated in our study had at least one child with a birth defect (10.1%; Table 6). Other than these crude comparisons, enumeration and determination of the frequency of families with at least one child with a birth defect has received little attention in the current available literature. The frequency of birth defects (structural, chromosomal, genetic, and metabolic disorders) in relation to the number of live births (76/1,532 or 5.0% [Table 1, all cases] and 58/1,532 or 3.8% [Tables 2 and 3, cases confirmed by medical

Table 6. Children with parent-reported birth defects and paternal pesticide use.[a]

Exposure category	Total no. applicators with children		No. applicators with children who have birth defects		OR	95% CI
	n	%	*n*	%		
Herb/insect/fume	39	7.28	6	15.38	2.27	0.85–6.08
Herb/insect/fung	182	33.96	21	11.54	1.70	0.79–3.66
Herb/insect	73	13.62	9	12.33	1.82	0.74–4.46
Other[b]	58	10.82	5	8.62	1.27	0.43–3.73
All four	66	12.31	5	7.58	1.12	0.38–3.29
Herb only	118	22.01	8	6.78	Referent	

Abbreviations: fume, fumigants; fung, fungicide; herb, herbicide; insect, insecticide. [a]Pesticide applicator exposure groups were defined by pesticide class use. In these comparisons, applicators who used herbicides but not other products were assigned to the referent group. The number of applicators who have children with and without birth defects are reported in the table according to exposure group. Approximately 15% of applicators who apply herbicides, insecticides, and fumigants have children with birth anomalies. More detailed analysis (see text) of specific fumigant use shows that applications of the fumigant phosphine were associated with a significant increase in adverse birth, developmental, and neurobehavioral sequelae (OR = 2.5; CI 1.22–5.05). [b]Any pesticide combination not listed.

Table 7. Sex and sex ratio of children born to fungicide applicator families: with and without birth defects.

Births	Male	Female	Sex ratio	Chi-square
All children from families:				
Where fungicides were applied	373	408	0.91	$p = 0.04$
Where no fungicides were applied	387	343	1.13	
Children with birth defects:				
From families where fungicides were applied	13	23	0.57	$p = 0.02$
From families where no fungicides were applied	22	12	1.80	

The sex ratios (SR) of live-born children with and without birth defects were compared according to paternal (pesticide applicator) use of fungicides. Significantly fewer male children with (SR = 0.65) birth anomalies ($p = 0.02$) and without (SR = 0.91) birth anomalies ($p = 0.02$) were born to families where the father applied fungicides (chi-square).

Table 8. Birth defects by season of conception.[a]

Season of conception	Total births	Births with congenital anomaly	Percent with anomaly
Fall	390	13	3.3
Winter	349	13	3.7
Spring	381	29	7.6*
Summer	377	15	4.0
Totals	1,497[b]	70	4.7

[a]The number of births and birth anomalies resulting from conceptions in each season were examined. Compared with all other seasons, conceptions in the spring led to significantly more children with birth defects. [b]Total number of self-reported births recorded giving specific date of birth. *Chi-square, $p = 0.002$.

records]) is somewhat higher than that reported from regional/national norms [2.68% in Iowa (*15*)] and the U.S. Department of Defense birth defect surveillance system (3.2%) (*16*). Both of these birth defect surveillance systems employ active retrieval to ascertain cases and require birth/death or medical record confirmation for inclusion into the registry. The differences in the overall frequency of birth defects we observed could reflect parent bias in reporting, medical underreporting, and notably in our study, the reticence of adult children (Tables 2 and 3) to allow investigation of their birth and developmental medical records. Anecdotal parent reports suggested that the adult child had concerns regarding privacy and/or health insurability.

Comparison (Table 2) of the data from our earlier birth record cohort study (*3*) with data from our current medical records-based cross-sectional study shows a relatively uniform increase in the overall reported rate of birth defects in the cross-sectional study. These data also offer a tentative suggestion that the relative rate of urogenital birth defects in the RRV may be constant through time.

Season of Conception and Birth Defects

From our earlier-reported cohort study of birth defects (*3*) in pesticide applicators throughout Minnesota, we uncovered a seasonal increase in the frequency of birth defects occurring only in the RRV region of our state. As stated and detailed in our earlier publication, the valley is a unique wheat, sugar beet, and potato crop-growing region with specific pesticide use requirements. Conceptions in spring, the time frame of herbicide applications, led to an increased frequency of birth defects. Our present cross-sectional study reconfirms these initial findings (Table 8). Interestingly, in our report discussing fetal losses in this study group (*4*), the frequency of first trimester miscarriages also peaks in spring. The repetition of the spring season's findings regarding birth defects and the concordance with the peak frequency of miscarriages are suggestive of an environmental nonmutagenic origin of these adverse reproductive effects. In reports by others, the lack of a consistent seasonal trend for birth defects overall (*17,18*) and for particular birth defects (*19,20*) strengthens the possible connection with a seasonal environmental exposure to herbicides or other environmental contaminants uniquely present in the RRV during spring but not in other seasons. For example, shifts in the trace contaminant levels in residential well water during the spring thaw could be a factor in our results.

Recurrence of Birth Defects

Recurrence of birth defects within our population suggests that a segment of our study group is at special risk (Table 4). Several studies demonstrate that persons with one child with a specific birth defect are much more likely to have a second child with the same or similar birth defect [Mueller and Schwartz (*6*), relative risk (RR) = 11.7; Lie et al. (*7*), RR = 7.6]. The reported relative risk for having a second child with a different birth defect was slightly increased [Lie et al. (*7*), RR = 1.5; Mueller and Schwartz (*6*), RR = 1.5]. Also reported in these two earlier studies, the relative risks of having a second child with any birth defect were, respectively, 2.4 and 1.9. Further, in the study by Basso et al. (*8*), 5.5% of women whose child had a birth defect gave birth to a second child with a birth defect sired by the same father. In our study, 12 families contributed 28 children with birth defects. Twelve of 54 families (22.2%) where the father was a pesticide applicator had more than one child with a birth defect. Part of the differences in frequency of recurrent birth defects between the present study and the earlier studies by other investigators (e.g., 5.5 vs. 22.2%) can be due to differences in study requirements. Alternatively, environmental factors might give greater expression to birth defect susceptibility. Larger-scale studies of families with recurrent birth defects are needed to define possible gene–environment interactions.

Sex and Sex Ratio of Children Born to Applicator Families

The reported sex and M/F sex ratio (Table 7) of children with no reported birth defects born to spouses of pesticides applicators (M/F sex ratio = 1.01) show a reduction in male births in the RRV compared with data from our earlier study of the general population of Minnesota (M/F sex ratio = 1.05). Davis et al. (*21*) reported a long-term sustained reduction in the ratio of male to female births (1.06) in several industrial countries (Denmark, Netherlands, Canada, and the United States). It is generally accepted that more male children are born with birth defects (*22*). Among children with birth defects, if fungicide use is considered, then the sex ratio of children with birth defects born to applicators is much reduced (M/F sex ratio = 0.57), whereas if the male parent (applicator) did not use fungicides, then the M/F sex ratio is 1.83 (p = 0.02). This striking male predominance in the occurrence of birth defects among applicators who did not use fungicides is similar to that in our earlier published work (*5*). In this earlier ecologic study, fungicide use could not be distinguished from chlorophenoxy herbicide use due to lack of data on individual subject use of these products. In the present, smaller-scale but more detailed study, fungicide use by the male pesticide applicator parent was associated with fewer male births, most significant among children with birth defects. Similarly, the spouse of the pesticide applicator who applied or used fungicides on the farmstead is at a significantly higher risk for a first trimester miscarriage (*4*). The majority of these fungicide applications cited were conducted by air.

The decline in the ratio of the number of male to female children born to families with paternal exposure to fungicides poses a number of mechanistic questions. First, it is well known (subject to some controversy), that men in some environmental or occupational settings who were exposed to dioxins, dibromochloropropane (a fumigant), or boron, father significantly fewer live-born male children (*21,23–26*).

Most recently, Sakamoto et al. (*27*) reported a reduction of M/F sex ratio in Minamata Bay fishermen and among patients with Minamata disease, a disease entity associated with significant exposure to the pollutant methylmercury. These data, coupled with an increase in the number of male stillbirths in the study population, led the authors to speculate that the male fetus may be more susceptible to pollutants/toxicants. James (*23*) and others (*21,24–26*) hypothesized that differences in sex ratio are in part due to differences in the testosterone/gonadotropin ratio and blood levels of these sex hormones in both parents at the time of conception.

Indeed, under certain chronic disease conditions, lower testosterone levels in males have been associated with increased numbers of female infants born to their spouse (*28*). Astolfi et al. (*29*) noted that differences in paternal human leukocyte antigen (HLA) genotype were associated with differences in testosterone levels. Lower testosterone levels seen together with certain paternal HLA phenotypes were associated with a high number of daughters. These aforementioned data imply that testosterone levels may be relatively stable during much of male reproductive life and may affect the sex of the child. Regarding the role of the female in determination of the sex of children, Jongbloet et al. (*30*) suggested that several hormonally related factors, including differences in the length of the follicular phase of the menstrual cycle and the degree of maturation of the oocyte, may lead to differential attrition of the male conceptus.

Aside from the putative hormonal connection to germ-cell maturation and sex determination of the offspring, and of equal concern, is the well-known role of X-linked

recessive mutation in the determination of the sex of viable offspring. The U.S. Environmental Protection Agency (U.S. EPA) has offered guidelines for the inclusion of sex-linked recessive lethal screening test in *Drosophila* as a means of identifying germline mutations due to pesticides and other products (*31*). The U.S. EPA estimates that some 800 gene loci on the X chromosome will yield a lethal mutation such that fewer male offspring (F_2 generation) result from initial toxicant treatment of the male *Drosophila*. The extent and general applicability of these reproductive toxicant studies in *Drosophila* to humans have not, to our knowledge, been examined in detail.

In humans, skewed frequency of X chromosome inactivation in female carriers of proposed X-linked lethal genes has been associated with recurrent spontaneous abortions (*32*). In modeling these data, Lanasa et al. (*33*) suggested that the female carrier undergoes selective inactivation of the normal X chromosome; the remaining abnormal active X chromosome in a conceptus would be aborted. Confirmatory studies by these investigators are consistent with the initial hypothesis (*34*). Fewer boy children were born to female carriers. On the other hand, rare X-linked mutations (carriers of X-linked juvenile retinoschisis [RS]) have been associated with increased number of male offspring (*35*). These investigators showed that RS1 gene expression was confined to the uterus and speculated that RS1 protein has a role in implantation.

To summarize, it appears that determination of the phenotypic sex of children in human populations can be altered by X-linked mutational effects in a maternal carrier. Similarly, alteration of the sex-determining region (SRY) of the Y chromosome affects mammalian sexual development (*36*). Altogether, events associated with sex chromosome mutation are known determinants of the sex of offspring (*22*).

Finally, whether fungicides play a hormonal role, exert a mutational effect, or are aneugenic in the determination of sex of offspring is uncertain. Triphenyl tin (TPT) and mancozeb, fungicides in use for the past 20 years or more and that are in common use in the RRV, induce apoptosis *in vitro*. TPT alone induced aneuploidy (*37,38*). TPT is also an inhibitor of aromatase (*39*), a P450 enzyme that catalyzes the conversion of testosterone to estrogen in males. With respect to mutagenesis, as stated above, there is little information to suggest that TPT is a significant mutagen (*40*).

Mancozeb, a member of the ethylene bisdithiocarbamate fungicide group, is weakly mutagenic but is a significant thyroid carcinogen in animals (*41,42*) and affects thyroid function in humans (*43*). Studies at the germ cell level, including the recessive lethal test in *Drosophila*, were negative (*44,45*).

Birth Defects

Division of the pesticide-exposed population according to pesticide use by class (Table 6) and examination of the frequency of birth defects, revealed an interesting but nonsignificant excess of birth defects among applicators who apply fumigants and other products (OR = 2.27; CI, 0.85–6.08). Inclusion of neurodevelopmental abnormalities led to a statistically significant result (OR = 2.5; CI, 1.2–5.1). As mentioned in "Results," phosphine derived from the phosphide was the major fumigant in use. This highly toxic, denser-than-air gaseous fumigant can be genotoxic (*46*). DNA damage expressed as increased DNA adducts (*47*) or excess chromosome breakage and rearrangement has been noted (*48*). In this connection, we recorded two cases of congenital cataracts from different families among 290 children born to 113 applicators who apply this fumigant. No antecedent family history of cataracts was noted in either family, nor was there history of pregnancy-associated diabetes or rubella (*49*). In the United States (Birth Defects Monitoring Program), the reported frequency of congenital cataracts is between approximately 1.1–2.2 per 10,000 live births (*50*). Specific studies of congenital ocular anomalies in the United Kingdom (*51*) put the frequency of this class of congenital defect up to 3.5 per 10,000 live births through age 15. It appears that chemical induction of this birth defect either through dominant or recessive mutation (*52–54*) or by direct chemical toxicity to the developing fetus (*55*) is a distinct possibility and concern. Based on the laterality pattern observed (right eye only) in both cases, germ-cell mutation seems more likely (*56*). In relation to occupation, paternal exposure to dioxin-contaminated chlorophenols in the sawmill setting has been associated with excess congenital cataracts in children born to these workers (*57*). Connection with the present study is tentative. Other laboratory-based studies examining possible dioxin content of chlorinated pesticides in current use are under way.

Again, we noted that the two cases of parent-reported autism clustered within the phosphine-use group. Current data (*58*) estimates the prevalence of autism somewhere between 1 and 2 per 1,000 in children less than 15 years of age, similar to the frequency reported in our entire study group (2 cases/1,532 live-born children) but highly prevalent in the paternal phosphine-use group (2 cases/290 live births). Genes involved in susceptibility to autism have been mapped to chromosome locus 7q32.3-q33 (*59,60*). The Online Mendelian Inheritance in Man (*61*) places a putative autism susceptibility region at 7q31. Giemsa-banded chromosome studies of phosphine-exposed workers performed by our group showed that breakage at these chromosome loci (7q31 and/or 7q32) was more common among phosphine-exposed workers than among control subjects (*12,48,62*).

Regarding phosphine, 5 of 14 parent-reported ADD/ADHD cases were recorded in this exposure group. Current reports of the prevalence of ADD/ADHD show much variability. A study of children from Manitoba, Canada (*63*), showed a prevalence rate of 1.5%. The combined prevalence of ADD/ADHD reported by Nolan et al. (*64*) in the United States was 3.5%; and Brown et al. (*65*) reported that the frequency varied from 4 to 12% of children. In the present study (Table 1), parents reported 14 cases among 1,532 live births, less than expected. However, the prevalence of ADD/ADHD among children of phosphine applicators (5 of 290, or 1.7%) is about the same as that in the Canadian studies.

Altogether, these neurologic, neurodevelopmental, and neurobehavioral data suggest that phosphine may affect the developing nervous system, perhaps at critical times (*66*) or by germ-cell mutation.

Regarding the herbicide glyphosate, our present study shows a tentative association between ADD/ADHD and use of this herbicide. *In vitro* studies by our group show that this product was not genotoxic in the micronucleus assay (*67*) and did not have significant pseudoestrogenic effects in MCF-7 cells (*37*). In a recent review of the toxicology of glyphosphate (*68*), little if any evidence of neurotoxicity was noted other than by intentional ingestion (*69*).

Finally, of the 14 pesticides identified by class (Table 5), only phosphine and glyphosphate showed a significant correlation with excess adverse birth and neurodevelopmental effects. Whether these observations were chance associations remains a concern. Further detailed neurodevelopmental studies are required to resolve these issues.

Summary

Previously, we showed that applicators and the general population of the RRV had the highest birth defect rate in the state of Minnesota (*3*). In the present more detailed study, we showed that in families of fungicide applicators from the RRV, the number of live-born male children with or without birth defects was significantly reduced. As in our earlier study, conceptions in the spring led to significantly more children with birth defects compared with children conceived in any other

season. These data suggest that environmental agents present in the spring, perhaps herbicides, have an adverse effect on the birth defect rate. A different class of agent, probably fungicides, selectively affects the survival of the male fetus. Aneuploid events and endocrine disruption alone or in combination are plausible biologic mechanisms for the adverse reproductive effects observed. Biologically based human, animal, and *in vitro* studies are needed to test these hypotheses.

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