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Pre- and post-conception pesticide exposure and the risk of birth defects in an Ontario farm population

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ABSTRACT

The use of pesticides has enhanced the health and economies of nations around the world by improving crop production. However, pesticides may pose health risks, particularly to the fetus and young children. In a secondary analysis of the Ontario Farm Family Health Study, we explored the relationship between birth defects and parental pesticide exposure during the 3 months prior to conception and the first trimester of pregnancy. A total of 3412 pregnancies were included in the study. Logistic regression fit by maximum likelihood was used in the analysis. The results showed that pre-conception exposure to both cyanazine (odds ratio = 4.99, 95% confidence interval: 1.63–15.27) and dicamba (OR = 2.42, 95% CI: 1.06–5.53) were associated with increased risk of birth defects in male offspring. Nevertheless, given the self-reported nature of the exposure and outcomes in this study, the present findings should be considered primarily as hypothesis generating, requiring verification in subsequent investigations.

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1. Introduction

Experimental studies have shown specific pesticides to increase the risk of several birth defects in rodents and amphibians including: cardiac anomalies [1], cleft palate [2], skeletal anomalies [3], cryptorchidism [4], hypospadias [4], retained nipples [5] and limb deformities [6]. The effect of pesticides on human fetal development, remains unclear. Since the 1960s, a number of epidemiological studies have examined birth anomalies in the offspring of parents exposed to pesticides, however inaccurate exposure assessments have plagued many of the studies in this area. Several studies have relied on job title only as a measure of exposure, or have used vague exposure measures such as "any pesticide" or proxy exposure measures. Only a few studies have employed biomarkers of exposure to pesticides in infants [7], mothers [8–11] or fathers [12] of infants with birth anomalies. Further, few epidemiologic studies have examined the effect of exposure to a specific pesticide class, family, or active ingredient during a critical exposure window on the risk of birth defects [13–17].

The objective of this study is to estimate the effect of parental pesticide exposures in the pre- and post-conception periods on the prevalence of birth defects in offspring.

2. Methods

The Ontario Farm Family Health Study (OFFHS) was conducted between 1990 and 1993 with the objective of retrospectively assessing the relationship between phenoxy herbicides and spontaneous abortion [18]. The methods used in the OFFHS have been described in detail elsewhere [19]. Briefly, the sampling frame consisted of all farm operations in Ontario reported in the 1986 Census. Farms were restricted to family run farms with reported sales of agricultural products of $50,000 or greater in 1986. Farms were excluded if they were: a legally constituted company with most of the shares owned by some other person(s) or business; institutions; community pastures; land operated privately for an estate or trust company; or cooperative farms. Tobacco farms were also excluded due to their small numbers and the distinct types of pesticides that they use [18].

All farms meeting these criteria were contacted in order to determine eligibility of the couples living on the farms. To be eligible for the study, couples had to be married or common-law, living year-round on the farm operation, and the wife had to be 44 years of age or younger. In total, 2946 eligible couples were identified.

Three questionnaires were mailed to each farm family in order to gather information on the family's health, pesticide use, and farm activity exposures. A farm operator form was used to collect information on farm operations, as well as present and former pesticide use on the farm. Another questionnaire was addressed to the husband and gathered information on basic demographic, socioeconomic, lifestyle, medical history, as well as his activities and chemical exposures on the farm. The

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final questionnaire was addressed to the wife and collected similar information to the husband's questionnaire, but also included a complete reproductive history of her first five pregnancies. The study was approved by the human subjects ethics committee at the University of North Carolina. The participants agreed to participate by phone and there is implied consent because they completed the voluntary questionnaire by mail or phone.

2.1. Birth defect data

As part of their reproductive histories, wives were asked to report on any pregnancies that resulted in a birth defect(s) diagnosed at or since birth. If a pregnancy did result in a birth defect, the respondent was asked to describe the birth defect(s). Birth defects were cataloged into appropriate ICD-9 codes by the authors (MW, DW, TA) and a maternal fetal medicine physician (MW). Analyses were conducted on pregnancies ending in one or more birth defect (n = 108), as well as musculoskeletal defects (ICD-9: 754.0–756.9) (n = 43).

2.2. Pesticide exposure information

2.2.1. Farm pesticide use

The farm operator provided detailed information on agriculture chemicals used on the farm's six largest crops sown or harvested in 1991. The crop name, chemical name, reason for use, months of application, and number of years of use were obtained. In addition to this, the farm operator provided information on historical chemical use including: any changes to the types of agricultural products grown or livestock raised since they started to work on this farm, any chemicals that they regularly used in previous years but have since stopped using, and any other chemicals that have been used on the farm. The husband and wife questionnaires also asked for information pertaining to other pesticide exposures on the farm. For each exposure, the name of the chemical (up to two), reason for its use, months of use, and method of application was requested.

2.2.2. Direct chemical activities

The husband and wife also completed a checklist relating to his/her farm operation activities over the past 5 years including the frequency (number of days), intensity (never, occasionally, regularly), and months during which they were involved in these activities. Farm activities that related to direct chemical activities were collapsed into one exposure variable and included mixing or applying chemicals to: (1) crops for weed or brush control; (2) crops for insect or disease control; (3) to livestock to control insects and disease; (4) around the yard and buildings to control weeds and brush; and (5) around the farm buildings to control pests. For the purposes of this analysis, the assumption will be made that the reported chemical activities extends beyond the past 5 years back to the time of the first pregnancy.

The pesticide use information from all three questionnaires was collapsed into a pesticide history file. Relevant chemical data from all the questionnaires was extracted to a pesticide history file where the active ingredient was identified for each pesticide product using a database of registered pesticide products in Canada. In this file, each observation corresponded to a pesticide product used on a farm. The active ingredient was then identified for each pesticide product using a database of registered pesticide products in Canada. The active ingredient and chemical families were identified for those pesticides that were most frequently used on the farm and those that had reported reproductive effects according to the literature [20]. Variables were created that matched the month and year of chemical use on the farm. An imputation process was used in cases where the dates of chemical exposure were missing. When the month or year of application of a particular pesticide was not reported, the exposure was considered to have occurred in a particular calendar month or year if at least 75% of all the applications of that pesticide within the study population reported use in that particular month or year. The year and month of chemical use was then matched with the months and years leading up to and of each pregnancy.

Seventeen different pesticide exposure variables were created that identified pregnancies with reported farm chemical use during the pre-conception and the post-conception period. Pre-conception exposures occurred if there was reported use of a particular pesticide class, family, or active ingredient on the farm in the 3 months prior to conception. Post-conception exposures involved the reported use of pesticides during the 3 months after conception. Unexposed pregnancies were those in which the farm did not report using the pesticide of interest during the 3 month periods pre- or post-conception. Similar methods were used to create exposure variables for fathers involved in farm chemical activities during these same time periods.

2.3. Statistical analysis

Using logistic regression fit by maximum likelihood, a base model was derived that included the primary exposure variable of interest, along with the following known risk factors for birth defects: maternal fever during pregnancy, sex of the offspring, maternal age at conception, and parity. Other potential covariates were identified and their crude odds ratios were calculated. Those covariates with odds ratios greater than 1.2 or less than 0.8 were added to the base model individually. If the addition of the covariate changed the exposure odds ratio by 10% or more, it was included in the final model [21]. Other potential covariates included: maternal and paternal age, education level, income, and ethnicity, maternal caffeine and alcohol consumption during pregnancy, maternal and paternal smoking during pregnancy, maternal fever, maternal medical problems and weight gain during pregnancy, labor and delivery problems, parity, season of conception, number of years of recall to the pregnancy, and the number of years the mother lived on the farm prior to conception. In order to allow for the possibility of familial correlations, final models were also run using generalized estimating equations (GEEs) with the "exchangeable" structure. This structure assumes that the correlation between subsequent pregnancies to be the same, irrespective of the length of time between pregnancies [22]. This resulted in virtually no change in the ORs indicating that the effect of intercluster or familial correlation was minimal.

3. Results

A total of 5853 pregnancies were identified by 1893 couples who responded to all three questionnaires. The following pregnancies were excluded from the analysis: 110 pregnancies with missing data on outcome, delivery data, or gestational age at delivery; 1649 pregnancies that occurred when the woman was not living on the study farm and was of unknown exposure status; 27 pregnancies for which the husband may not be the father (because the mother reported more than one marriage and the pregnancy occurred more than 1 year before the date of marriage to the husband); and 395 miscarriages, 31 stillbirths, 13 induced abortions, 36 ectopic pregnancies, 5 hydatidaform moles, and 118 current pregnancies. In addition, 15 pregnancies were excluded because the mother did not answer the question relating to birth defects leaving 3412 pregnancies for the analysis. When analyzing musculoskeletal defects only, the 65 pregnancies ending in other birth defects were excluded from the analysis, leaving a total of 3347 pregnancies.

There were 118 birth defects identified from 108 pregnancies (3.17%), with 10 pregnancies (0.28%) ending in multiple birth defects (see Table 1). The majority of the birth defects were musculoskeletal (37%), followed by defects of the integument (17%) and heart (11%). The percentage of male (50.4%) and female (49.6%) offspring included in the study were nearly equal. The mean age of women was 26.3 years (median age 26 years) at the time of conception. Men (mean age 29.0 years, median age 28 years) were slightly older than women. More women (48.7%) than men (36.1%) had some form of post-secondary education, most mothers (90.4%) and fathers (98.4%) were of European descent, and the median family income was $35,000. Women in the study had lived on the farm for a median of 3 years prior to the year of conception year, with a wide range of 0–36 years. The median number of years since the pregnancy was 8 years at the time of the questionniare, with a range of 0–27 years.

The bulk of the chemical data (89%) utilized for exposure assessment in the present analysis came from the Farm Operator Survey

Table 1
Type and frequency of birth defects

Category	ICD-9 code	Frequency	%
Face and neck	744.0–744.9	1	0.8
Chromosomal	758.0–758.8	4	3.4
Digestive	750.0–751.9	15	12.7
CNS	740.0–742.9	6	5.1
Cleft lip/palate	749.0–749.2	6	5.1
Urogenital	752.6–753.9	9	7.6
Heart	745.0–746.9	13	11.0
Integument	757.0–757.9	20	16.9
MUSC	754.0–756.9	44	37.3
Total malformations		118	100.0
Total infants with multiple malformations[a]		10	
Total infants with birth defects		108	

[a] 10 infants had 2 malformations each.

(Form A). Only 53% of the husbands and 5.9% of the wives indicated that they were the farm operators. The majority of men (81.7%) and most women (62.9%) did not work off the farm during the pregnancy. For most (57.9%) of the pregnancies, use of some type of pesticide on the farm during the pre- or post-conception periods was reported. Over half of the fathers reported direct chemical activity during both the pre- (59.1%) and post-conception (52.3%) periods. Conversely, only 2.5% of the women reported being involved in chemical activites during these same periods. In the pre-conception period, the most frequently reported pesticide class was herbicides (27%), while in the post-conception period insecticides (22%) were most often reported. Phenoxy herbicides were the most common pesticide family used in both the pre- and post-conception periods (15% and 11%, respectively), and 2,4-D was the most common active ingredient (9% and 6%).

The distribution of other potential risk factors is given in Table 2. The crude risk of self-reported birth defects was found to be increased in women who drank 3 or more cups of tea or coffee in the first trimester, and among those who had a fever during pregnancy. An increased risk was also seen for the first pregnancy (parity = 1), male offspring, and women who lived less than 1 or 1–4 years on the farm, as compared to women who had lived on the farm for more than 4 years. Birth defects did not appear to be associated with parental age, education, income, alcohol intake, or smoking.

3.1. Any birth defect

3.1.1. All offspring

No statistically significant associations were observed between reported pesticide use during the pre-conception period and birth defects in offspring. However, some risk estimates were numerically elevated, including: dicamba (OR = 1.67, 95% CI: 0.79–3.53), and cyanazine (OR = 2.31, 95% CI: 0.81–6.57) (see Table 3). In the post-conception period, there were no statistically significant associations between reported pesticide use and birth defects (see Table 3).

3.1.2. Stratification by gender

Gender specific results for male (Table 4) and female (Table 5) offspring showed significantly elevated adjusted odds ratios for male offspring in relation to reported use of dicamba (OR = 2.42, 95% CI: 1.06–5.53) and cyanazine (OR = 4.99, 95% CI: 1.63–15.27) in the pre-conception period. In the post-conception period reported use of fungicides, thiocarbamate, and organophosphates all demonstrated odds ratios in excess of 1.60. No significant relationships were observed in female offspring. In fact, for female births, herbicide exposure in the pre-conception period was associated with a reduced risk of any birth defect (OR = 0.36, 95% CI: 0.14–0.93).

3.2. Direct chemical activity by father

An additional exposure group was created to examine couples who lived on farms where the father had reported direct chemical activity during a relevant period of time, and there was reported use of a particular pesticide class, family, or active ingredient during that same time period (too few mothers reported being involved in chemical activities for analysis). It should be noted that this combination of chemical activity and farm chemical use does not necessarily mean that the father mixed or applied that type of pesticide: this method of exposure assessment was employed because there was no direct linkage in the questionnaire between farm chemical activities and the type of pesticide mixed or applied.

No association was observed between a father's involvement in chemical activities and birth defects in the pre-conception period. There was a non-significant elevation in risk in offspring whose fathers' were involved in chemical activities and reported use of cyanazine (OR = 2.26, 95% CI: 0.75–6.77). Other combinations of exposures were non-significant (Table 6). No significant associations were observed for chemical activities in the post-conception period.

3.3. Musculoskeletal defects

The risk of musculoskeletal defects (ICD-9: 754.0–756.9) in relation to parental pesticide exposure was also explored. Overall, no association between reported farm chemical use in the pre- or post-conception periods and musculoskeletal defects was observed. However, reported use of fungicides (OR = 2.29, 95% CI: 0.89–5.93) in the post-conception period did have numerically elevated risk odds ratios, but with wide confidence intervals that included the null value of unity (Table 7).

4. Discussion

There were no statistically significant associations between reported pesticide use in the pre- or post-conception periods and birth defects in male and female offspring combined. However, reported use of the herbicides cyanazine (OR = 4.44, 95% CI = 3.66–4.99) and dicamba (OR = 2.42, 95% CI = 1.07–5.47) in the pre-conception period were associated with a significant increase in birth defects among male offspring. Cyanazine is an active ingredient from the triazine chemical family [23] and is used for early pre-plant, pre-emergence or post-emergence weed control of corn, cotton, grain, sorghum and fallow cropland [24]. Experimental studies have observed an increase in eye malformations (microphthalmia, anophthalmia) among rats exposed to cyanazine during gestation. These effects may be a result of direct toxicity to the fetus, and not simply a consequence of maternal toxicity [24].

Dicamba is a broad spectrum benzoic acid herbicide used for general weed control on grain crops, seed crops, pastures and non-crop areas [23]. Cavieres et al. [25] did not observe fetotoxicity among the pups of mice exposed to a common commercial herbicide formulation containing a mixture if 2,4-D, mecoprop, dicamba, and inactive ingredients. More recently, Greenlee et al. [26] showed that incubating mice embryos with dicamba significantly increased the percentage of apoptosis.

No other epidemiological studies have looked specifically at the effects of cyanazine or dicamba on birth defects; however, some studies have shown an increased risk of birth defects among the offspring of parents with potential unspecified herbicide exposures [16,27,28]. Parental exposure to triazine herbicides has been shown to increase the risk of birth defects [29,30], pre-term labor [31] and intrauterine growth retardation [32]. However, cyanazine itself showed no effect on intrauterine growth restriction [32] or fetal death [20].

4.1.1. Pre-conception exposures

Exposures occurring in the pre-conception period are most likely acting on spermatogenesis in the father. Studies on workers occupationally exposed to a mixture of pesticides, including cyanazine, have shown some evidence of genotoxic effects [33,34]. Furthermore, experimental studies indicate that spermatozoa with DNA damage are still able to fertilize the oocyte, providing a mechanism for male-mediated reproductive effects [35]. Other epidemiologic studies examining the effect of paternal pesticide exposures during the pre-conception period have shown an increased risk among the offspring of fathers exposed to pyridil derivatives [15] and chlorophenate wood preservatives [13]. As well, fathers who worked in agriculture during this time period

Table 2
Birth defect odds ratios in relation to risk factors

Risk factor	No. of cases	Total pregnancies	Percent pregnancies	Crude OR	95% CI
Maternal age					
<25.0	42	1183	(34.7)	1.00	
25–29	48	1526	(44.7)	0.88	0.58, 1.34
>30.0	18	703	(20.6)	0.71	0.41, 1.25
Paternal age					
<25.0	20	705	(20.7)	1.00	
25–29	50	1417	(41.5)	1.25	0.74, 2.12
>30.0	38	1290	(37.8)	1.04	0.60, 1.80
Maternal education					
<High school	10	473	(13.9)	0.63	0.32, 1.21
≥High school	98	2936	(86.1)	1.00	
Paternal education					
<High school	28	996	(31.2)	0.81	0.52, 1.25
≥High school	76	2195	(68.8)	1.00	
Income					
≤$25000	47	1280	(44.1)	1.17	0.72, 1.89
$25000.01–$45000	26	767	(26.4)	1.08	0.62, 1.86
>$45000	27	856	(29.5)	1.00	
Alcohol in 1st trimester[a]					
Yes	8	213	(6.2)	1.21	0.58, 2.52
No	100	3199	(94.8)	1.00	
Smoked in 1st trimester[b]					
Yes	13	516	(15.1)	0.76	0.42, 1.37
No	95	2896	(84.9)	1.00	
Maternal fever in pregnancy					
Yes	9	158	(4.7)	1.91	0.95, 3.85
No	98	3192	(95.3)	1.00	
Parity					
Parity = 1	41	964	(28.3)	1.70	1.07, 2.69
Parity = 2	32	1074	(31.5)	1.18	0.72, 1.91
Parity ≥ 3	35	1374	(40.3)	1.00	
Weight gain (lbs)[c]					
<20	11	362	(11.2)	1.00	
20–40	64	2239	(69.0)	0.76	0.47, 1.23
>40	24	646	(19.9)	0.81	0.39, 1.68
Child's gender					
Male	64	1719	(50.4)	1.45	0.98, 2.14
Female	44	1693	(49.6)	1.00	
Years on farm[d]					
0–1 year	39	902	(26.4)	2.03	1.21, 3.40
>1–4 years	45	1408	(41.3)	1.48	0.90, 2.45
>4 years	24	1102	(32.3)	1.00	
Length of recall since delivery					
0–5 years	38	1233	(36.1)	0.93	0.59, 1.48
>5–10 years	33	1053	(30.9)	0.95	0.59, 1.53
>10 years	36	1090	(31.9)	1.00	

[a] Drank 3 or more alcoholic drink in 1st trimester.
[b] Smoked 1+ cigarettes per day in 1st trimester.
[c] During pregnancy.
[d] Prior to conception.

had an increased prevalence of cleft lip or palate [36], neural tube [37], developmental [38], limb-reduction, central nervous system, and urogenital defects [39] in their offspring.

4.1.2. Direct chemical activity

There was no evidence that paternal involvement in chemical activities (mixing or applying chemicals), coupled with the reported use of pesticides in the pre- or post-conception period, was associated with birth defects in offspring. This combined exposure measure is considered to be a more accurate measure of exposure for this dataset. However, most risk estimates remained insignificant or moved closer towards the null (see Table 6), as compared to risk estimates that just reported farm use of a particular pesticide during the pre- or post-conception period (see Table 3); suggesting no association with paternal pesticide exposure and birth defects, or that this was not an improved exposure measure.

4.1.3. Male fetus susceptibility

No increase in risk was observed in female offspring from reported use of pesticides in the pre-conception and post-conception periods. Our data also demonstrated a noticeably higher percentage of exposed male verses female cases. This finding may

Table 3
Reported farm chemical use and any birth defect (all offspring)

		Exposed		Unexposed[a]		Adjusted MLE OR[b]	95% CI	Adjusted GEE OR[c]	95% CI	Adjustment[d]
		Cases	Total	Cases	Total					
Pre-conception										
Herbicides	Class	24	917	84	2495	0.65	0.40, 1.07	0.66	0.41, 1.07	i
Fungicides	Class	18	403	90	3009	1.33	0.75, 2.34	1.29	0.70, 2.40	i
Insecticides	Class	26	899	82	2513	0.73	0.45, 1.19	0.74	0.46, 1.19	i
Other pesticides	Misc.	8	272	100	3140	0.58	0.23, 1.47	0.57	0.24, 1.36	ii, iii
Phenoxy	Family	12	522	96	2890	0.6	0.32, 1.13	0.61	0.32, 1.14	–
Triazines	Family	9	381	99	3031	0.63	0.30, 1.31	0.64	0.31, 1.31	–
Organophosphates	Family	12	347	96	3065	0.94	0.48, 1.82	0.95	0.50, 1.81	i
Thiocarbamates	Family	7	201	101	3211	1.02	0.44, 2.38	1.01	0.44, 2.30	i
Carbaryl	AI	7	285	101	3127	0.65	0.28, 1.51	0.67	0.30, 1.48	i
Cyanazine	AI	5	71	103	3341	2.31	0.81, 6.57	2.22	0.75, 6.52	i
2,4-D	AI	10	296	108	3116	1.07	0.55, 2.08	1.06	0.55, 2.02	–
Dicamba	AI	8	158	100	3254	1.67	0.79, 3.53	1.65	0.76, 3.55	–
FUNG/INSC	Mixture[e]	15	334	67	2003	1.14	0.60, 2.16	1.15	0.58, 2.27	i
HERB/INSC	Mixture	12	450	67	2003	0.59	0.29, 1.22	0.60	0.29, 1.21	i
HERB/FUNG	Mixture	8	220	67	2003	0.85	0.36, 2.01	0.84	0.35, 2.03	i
Post-conception										
Herbicides	Class	7	395	101	3017	0.53	0.24, 1.15	0.53	0.25, 1.25	–
Fungicides	Class	17	355	91	3057	1.52	0.86, 2.69	1.51	0.87, 2.62	i
Insecticides	Class	22	751	86	2661	0.76	0.45, 1.28	0.77	0.46, 1.27	i, iv
Other Pesticides	Misc.	10	253	98	3159	1.10	0.53, 2.31	1.10	0.53, 2.29	iii
Phenoxy	Family	9	376	99	3036	0.77	0.39, 1.54	0.76	0.38, 1.50	–
Triazines	Family	9	282	99	3130	0.99	0.50, 2.00	1.03	0.53, 2.00	–
Organophosphates	Family	12	285	96	3127	1.14	0.58, 2.23	1.14	0.60, 2.17	i
Thiocarbamates	Family	7	167	101	3245	1.37	0.62, 3.00	1.36	0.62, 2.96	–
2,4-D	AI	7	210	101	3202	0.97	0.42, 2.25	0.94	0.40, 2.18	i
FUNG/INSC	Mixture	13	303	80	2451	1.11	0.58, 2.14	1.13	0.61, 2.10	i

[a] All offspring exposed to that particular pesticide in the exposure window compared to pregnancies not exposed to that pesticide.
[b] Adjusted OR using maximum likelihood estimate (MLE). All models contain a priori variables including: mother's age at conception, maternal fever during pregnancy, child's gender and parity.
[c] Adjusted OR using generalized estimating equations (GEE) estimate.
[d] Covariates that changed the exposure odds ratio by 10% or more when added to the base model. (i) Income; (ii) no. of years of recall since the pregnancy; (iii) maternal weight gain during pregnancy; (iv) maternal BMI at the time of the questionnaire.
[e] Mixture means exposure to both pesticide classes during the exposure window compared to pregnancies not exposed to herbicides, insecticides, or fungicides in the time window.

have been due to underreporting of birth defects in female offspring or an over-reporting of farm chemicals used among farm families who had sons verses farm families who had daughters. Alternatively, it may be that male offspring are differentially susceptible to *in utero* agricultural chemical exposures. Bradley et al. [40] found an increase in craniosynostosis in male offspring when fathers worked in agriculture or forestry for at least 10 h a week during the 3 months prior to conception (OR = 10.4, 95% CI: 2.3–46.5). Schreinemachers

Table 4
Reported farm chemical use and any birth defect (male offspring)

	Exposed		Unexposed		Adjusted MLE OR[a]	95% CI	Adjusted GEE OR[b]	95% CI	Adjustment[c]
	Cases	Total	Cases	Total					
Pre-conception									
Fungicides	13	192	51	1527	1.55	0.77, 3.14	1.53	0.73, 3.19	i
Insecticides	20	447	44	1272	0.96	0.53, 1.73	0.97	0.55, 1.71	i
Herbicides	19	470	45	1249	0.88	0.49, 1.59	0.88	0.48, 1.59	i
Phenoxy Herbicides	9	259	55	1460	0.78	0.36, 1.67	0.75	0.34, 1.67	–
Triazine	7	191	57	1528	0.83	0.35, 1.97	0.79	0.32, 1.95	–
Thiocarbamate	6	100	58	1619	1.47	0.57, 3.81	1.45	0.57, 3.68	i
Organophosphates	10	172	54	1547	1.32	0.61, 2.86	1.35	0.66, 2.80	i
2,4-D	7	153	57	1566	1.25	0.56, 2.81	1.25	0.55, 2.84	–
Dicamba	7	87	57	1632	2.42	1.06, 5.53	2.34	0.97, 5.67	–
Cyanazine	5	36	59	1683	4.99	1.63, 15.27	4.62	1.35, 15.79	i
Post-conception									
Fungicides	11	168	53	1551	1.61	0.77, 3.38	1.56	0.73, 3.34	i
Insecticides	15	365	49	1354	0.91	0.47, 1.75	0.91	0.48, 1.73	i, iii
Other Pesticides	5	133	59	1586	0.88	0.31, 2.48	0.89	0.32, 2.44	ii
Triazine	6	155	58	1564	1.04	0.44, 2.46	1.09	0.48, 2.50	–
Thiocarbamate	5	82	59	1637	1.71	0.67, 4.41	1.63	0.64, 4.14	–
Organophosphates	9	140	55	1579	1.62	0.74, 3.52	1.60	0.75, 3.42	i

[a] Adjusted OR using maximum likelihood estimate (MLE). All models contain a priori variables including: mother's age at conception, maternal fever during pregnancy, child's gender and parity.
[b] Adjusted OR using generalized estimating equations (GEE) estimate.
[c] Additional covariates that changed the exposure odds ratio by 10% or more when added to the base model. (i) Income; (ii) maternal weight gain during pregnancy; (iii) maternal BMI at the time of the questionnaire.

Table 5
Reported chemical use and any birth defect (female offspring)

	Exposed		Unexposed		Adjusted[a] MLE OR	95% CI	Adjusted[b] GEE OR	95% CI	Adjustment[c]
	Cases	Total	Cases	Total					
Pre-conception									
Fungicides	5	211	39	1482	1.00	0.38, 2.62	1.01	0.39, 2.57	i
Insecticides	6	452	38	1241	0.44	0.18, 1.07	0.92	0.18, 1.04	i
Herbicides	5	447	39	1246	0.36	0.14, 0.93	0.36	0.14, 0.92	i
Post-conception									
Fungicides	6	187	38	1506	1.50	0.61, 3.71	1.50	0.62, 3.62	i
Insecticides	7	386	37	1307	0.58	0.24, 1.42	0.58	0.25, 1.37	i, iii
Other Pesticides	5	120	39	1573	1.46	0.50, 4.25	1.46	0.51, 4.20	ii
Phenoxy	5	195	39	1459	1.12	0.43, 2.92	1.13	0.44, 2.90	

[a] Adjusted OR using maximum likelihood estimate (MLE). All models contain a priori variables including: mother's age at conception, maternal fever during pregnancy, child's gender and parity.
[b] Adjusted OR using generalized estimating equations (GEE) estimate.
[c] Covariates that changed the exposure odds ratio in the base model by 10% or more when added to the base model. (i) Income; (ii) maternal weight gain during pregnancy; (iii) maternal BMI at the time of the questionnaire.

Table 6
Direct chemical activity and any birth defect (all offspring)

	Exposed[a]		Unexposed		Adjusted MLE OR[b]	95% CI	Adjusted GEE OR[c]	95% CI	Adjustment[d]
	Cases	Total	Cases	Total					
Pre-conception									
Chemical activity[e]	61	2018	47	1394	0.70	0.46, 1.05	0.70	0.46, 1.05	i, iii
Fungicides	13	337	42	1328	0.86	0.42, 1.76	0.85	0.41, 1.76	i, iii
Insecticides	20	711	41	1206	0.57	0.31, 1.05	0.57	0.31, 1.04	i, iii
Herbicides	19	785	42	1262	0.53	0.29, 0.96	0.52	0.28, 0.96	i, iii
Triazine	8	342	47	1592	0.48	0.21, 1.11	0.49	0.21, 1.10	i, iii
Phenoxy herbicides	8	458	43	1330	0.42	0.18, 0.94	0.41	0.18, 0.95	iii
Organophosphates	10	294	45	1341	0.65	0.29, 1.42	0.65	0.30, 1.44	i, iii, iv
2,4-D	6	256	43	1354	0.60	0.25, 1.46	0.60	0.87, 1.43	i, iv
Cyanazine	5	63	47	1386	2.26	0.75, 6.77	2.19	0.72, 6.65	i, iv
Thiocarbamate	6	167	46	1360	0.84	0.32, 2.18	0.81	0.31, 2.12	i, iii
Post-conception									
Chemical activity	58	1783	50	1629	0.84	0.55, 1.29	0.84	0.55, 1.29	ii
Fungicides	12	305	45	1579	1.00	0.47, 2.13	1.00	0.47, 2.15	i, ii
Insecticides	14	583	42	1461	0.59	0.30, 1.18	0.59	0.29, 1.20	i, ii
Herbicides	6	329	49	1563	0.48	0.19, 1.23	0.49	0.19, 1.25	ii
Triazine	6	245	46	1355	0.77	0.30, 1.99	0.78	0.30, 2.01	ii, v
Phenoxy herbicides	6	314	47	1567	0.50	0.19, 1.28	0.50	0.20, 1.28	i, ii
Organophosphates	9	250	47	1594	0.83	0.36, 1.88	0.81	0.36, 1.85	i, ii

[a] Fathers who had direct exposure (i.e. mixing or applying chemicals on the farm) and reported farm chemical use during the exposure window compared to pregnancies where the father was not involved in chemical activities and there was no reported use of that type of chemical.
[b] Adjusted OR using maximum likelihood estimate (MLE). All models contain a priori variables including: mother's age at conception, maternal fever during pregnancy, child's gender and parity.
[c] Adjusted OR using generalized estimating equations (GEE) estimate.
[d] Covariates that change the exposure odds ratio (pesticide) by 10% or more when added to the base model. (i) Income; (ii) maternal weight gain during pregnancy; (iii) father's education level; (iv) father's age at the time of the pregnancy; (v) no. of years mother lived on the farm before the pregnancy.
[e] Any chemical activity vs. no chemical activity in the exposure window.

Table 7
Reported chemical use and musculoskeletal defects (all offspring)

	Exposed		Unexposed		Adjusted[a] MLE OR	95% CI	Adjustment[b] GEE OR	95% CI	Adjustment[c]
	Cases	Total	Cases	Total					
Pre-conception									
Herbicides	8	901	35	2411	0.49	0.20, 1.18	0.49	0.20, 1.18	i, ii, iii
Fungicides	5	385	38	2919	0.90	0.32, 2.58	0.84	0.27, 2.56	i, ii
Insecticides	8	881	35	2431	0.50	0.21, 1.22	0.52	0.23, 1.20	i, ii, iii
Other Pesticides	5	264	38	3040	0.55	0.13, 2.37	0.51	0.11, 2.28	ii, iv
Post-conception									
Fungicides	8	346	35	2966	2.29	0.89, 5.93	2.14	0.76, 6.02	i, ii, iii
Insecticides	10	739	33	2575	0.98	0.44, 2.19	0.97	0.45, 2.12	i, ii, iii
Other Pesticides	5	243	38	3061	1.02	0.31, 3.38	0.97	0.29, 3.29	i, ii, iii
Organophosphates	5	278	38	3069	1.42	0.49, 4.13	1.44	0.53, 3.88	i, ii, iii, iv

[a] Adjusted OR using maximum likelihood estimate (MLE). All models contain a priori variables including: mother's age at conception, maternal fever during pregnancy, child's gender and parity.
[b] Adjusted OR using generalized estimating equations (GEE) estimate.
[c] Covariates that change the exposure odds ratio (pesticide) by 10% or more when added to the base model. (i) Income; (ii) maternal weight gain during pregnancy; (iii) father smoking during the pregnancy; (iv) number of years of recall.

[41] observed an increase in circulatory/respiratory (OR = 1.83, 95% CI: 1.06–3.14), "other" circulatory/respiratory (OR = 2.05, 95% CI: 1.02–4.09), and all circulatory/respiratory defects (OR = 1.83, 95% CI: 1.06–3.14) in male offspring in areas of high as compared to low wheat acreage. Other studies have shown an increase in the risk of birth defects specific to males, including hypospadias and cryptorchidism, among parents potentially exposed to pesticides [39,42–44]. However, at this time we are unable to conclude with certainty whether these or other factors are responsible for the differences seen.

4.1.4. Musculoskeletal defects

No significant associations were observed between reported pesticide use in the pre- or post-conception periods and musculoskeletal defects. However, the odds ratio for musculoskeletal defects in relation to fungicide exposure in the post-conception period was numerically elevated (OR = 2.29, 95% CI: 0.89–5.93). Other research has shown significant elevations in musculoskeletal defects among women employed in agriculture or horticulture for 15 or more hours a week at the time of conception [45]. Also, an increased risk of musculoskeletal/integument defects (ICD-9: 754–757) was observed among families living in high verse low wheat regions (OR = 1.50, 95% CI: 1.06–2.12) [46], and in the wheat/sugar beets region (OR = 1.75, 95% CI: 1.4–2.2) [29] relative to urban regions in Minnesota. The wheat/sugar beets/potatoes region was considered to be a high pesticide use region based on poundage of fungicides and chlorophenoxy herbicides.

4.2. Study limitations

4.2.1. Reliability of the exposure assessment

The reliability of our indicators of exposure may have been affected by the retrospective nature of this study, and the fact that the recall period of pesticide use often exceeded 10 years in approximately 30% of pregnancies. As well, more than 10% of the values were imputed for some pesticide groupings. The reliability of self-reported exposure information from pesticide applicators was recently examined by Garry et al. [47], who found that the relative frequency of commonly applied pesticides was nearly identical for pesticide usage reported in a phone survey and that reported in a subsequent self-reported written survey carried out 6 months later.

The validity of the exposure assessment is also limited by a number of unmeasured factors, including quantity of pesticides used, time spent applying pesticides, and use of protective equipment which may have modified actual exposures. Also, since we did not account for pesticide half-lives, the unexposed group may have been inadvertently exposed due to the persistence of the pesticides in the farm environment after application.

Although pesticide active ingredients were identified, it is unknown if these groupings are relevant to reproductive toxicity. Most pesticides also contain carrier substances added to the active ingredients in order to improve absorption. These so-called inert substances, or contaminants introduced during processing, may be more harmful than the active ingredients [48]. We were also unable to account for possible additive or synergistic effects among inert substances or contaminants.

It was not possible in this study to completely separate the effects of maternal and paternal exposures. For birth defects, we assumed that exposures occurring in the pre-conception period were acting on spermatogenesis, while maternal exposures were more relevant in the first trimester of pregnancy. Pesticide residues have been measured in human semen [49,50], and may have been passed to the woman during intercourse. Therefore, paternal exposures in the post-conception period may have been relevant. Also, depending on the half-life of the pesticide, maternal exposures occurring in the pre-conception period may have been of importance.

4.2.2. Recall bias

Differential recall bias would have occurred if mothers or fathers of malformed infants recalled their exposures more thoroughly than mother's of healthy infants or children [51]. It is unlikely that there was differential exposure misclassification in this study because the majority of exposure information came from the farm operator, and women reported on reproductive outcomes. The farm operator reported on the years and months of use of farm chemicals, and was not made aware of the critical period of exposure to the fetus. However, we cannot rule out recall bias since those fathers who were the farm operator (50%) and had a child with a birth defect may have recalled the chemicals that they used on the farm more accurately than those who did not.

4.2.3. Limitations to the outcome assessment

Although birth defects were reported by the mother and not the father, self-reported outcomes may introduce bias due to erroneous recall. Other limitations included the ambiguity of the outcome measures (any birth defect). The use of the category "any congenital anomaly" as an outcome measure has been criticized for its vagueness [52]. Furthermore, Olsen et al. [53] suggest that disease classification is not based on principles associated with causal research [52]. Our results are further limited by the fact that we were unable to search medical records to confirm the birth defects.

Other challenges relate to missed birth defects. Since the prevalence of birth defects was derived from fetuses that survived until birth, malformations that were part of syndromes incompatible with survival of the fetus, or those electively aborted due to prenatal screening, are not represented in this analysis [53]. Consequently, the true incidence of birth anomalies may be higher than the prevalence of such anomalies at birth.

4.2.4. Other limitations

Other factors such as unmeasured confounders or selection bias may have affected the findings. Although the participation rate was fairly high (64%), those that agreed to participate may have been more concerned about their health, or may have had more family health problems than those who did not participate. It is also important not to over interpret our results. As the number of statistical tests conducted on a dataset rises, so does the probability of falsely rejected the null hypothesis solely due to chance [54]. One would therefore expect a number of "statistically significant" associations to occur by chance alone based on the alpha level of 0.05. Finally, given the modest sample size, the power to detect an association was low for rare outcomes such as birth defects.

5. Conclusions

In conclusion, this study is one of only a few to examine the human health effects of specific pesticide exposures during the pre- and post-conception periods. In general, our study did not find strong evidence for an association between parental pesticide exposure during the pre- or post-conception periods and birth defects among the offspring of Ontario farm families. There was some indication that pre-conception exposure to cyanazine and dicamba may increase the risk of birth defects in male offspring. However, given the large number of parameters estimated and the low number of pregnancies exposed in these two categories, it is possible that this association was due to chance. Since animal studies have shown that cyanazine and other triazines can induce birth defects, however, this association should be further investigated.

Several environmental chemicals with known adverse health effects have been found in umbilical cord blood [55], amniotic fluid [56,57], and meconium [58]. The impact of the *in utero* environment on the later health of the child and adult [59,60] is becoming increasingly recognized, reinforcing the need for a large well-designed prospective longitudinal study to examine the effect of environmental contaminants on reproductive outcomes.

Conflict of interest

None.

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