An Exploratory Analysis of the Effect of Pesticide Exposure on the Risk of Spontaneous Abortion in an Ontario Farm Population

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The toxicity of pesticides on human reproduction is largely unknown—particularly how mixtures of pesticide products might affect fetal toxicity. The Ontario Farm Family Health Study collected data by questionnaire on the identity and timing of pesticide use on the farm, lifestyle factors, and a complete reproductive history from the farm operator and eligible couples living on the farm. A total of 2,110 women provided information on 3,936 pregnancies, including 395 spontaneous abortions. To explore critical windows of exposure and target sites for toxicity, we examined exposures separately for preconception (3 months before and up to month of conception) and postconception (first trimester) windows and for early (< 12 weeks) and late (12–19 weeks) spontaneous abortions. We observed moderate increases in risk of early abortions for preconception exposures to phenoxy acetic acid herbicides [odds ratio (OR) = 1.5; 95% confidence interval (CI), 1.1–2.1], triazines (OR = 1.4; 95% CI, 1.0–2.0), and any herbicide (OR = 1.4; 95% CI, 1.1–1.9). For late abortions, preconception exposure to glyphosate (OR = 1.7; 95% CI, 1.0–2.9), thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) was associated with elevated risks. Postconception exposures were generally associated with late spontaneous abortions. Older maternal age (> 34 years of age) was the strongest risk factor for spontaneous abortions, and we observed several interactions between pesticides in the older age group using Classification and Regression Tree analysis. This study shows that timing of exposure and restricting analyses to more homogeneous endpoints are important in characterizing the reproductive toxicity of pesticides. *Key words*: atrazine, carbaryl, developmental toxicity, epidemiologic methods, glyphosate, herbicides, pesticides, phenoxy acetic acid herbicides, spontaneous abortion, thiocarbamates, triazine, windows of vulnerability. *Environ Health Perspect* 109:851–857 (2001). [Online 14 August 2001]
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Farm residents may be exposed to several types of pesticides from various chemical families (e.g., phenoxy acetic acids, triazines, carbamates, and organophosphates) during the course of a growing season. Several studies have reported positive associations between occupational pesticide exposure and fetal death (spontaneous abortion or stillbirth) (*1–3*). However, little is known about the human reproductive toxicity of specific pesticide active ingredients and even less about mixtures of pesticides and how they may interact with other risk factors.

In addition to the nature of the chemical and its target, the consequences of exposure to chemical agents depend on the timing of exposure relative to critical windows in development of the fetus or reproductive system (*4,5*). In a recent article (*6*), we noted that the risk of spontaneous abortion in farm families varied depending on when exposure to phenoxy herbicides occurred and on whether the abortion occurred earlier (< 12 weeks) or later (12–19 weeks) in the pregnancy. Previous analyses had also discussed the role of male pesticide exposure on pregnancy outcomes (*7*) and time to pregnancy (*8*). In this analysis we used the data from our study of farm families to explore further the critical windows of exposure, the target sites and interaction among the pesticides, and other risk factors for spontaneous abortion.

Subjects and Methods

The Ontario Farm Family Health Study collected data retrospectively by questionnaire from farm operators and eligible couples living on the selected farms, as described in detail elsewhere (*6,9*). To be eligible, the couple had to be living year round on the study farm and the wife had to be 44 years of age or younger (to reduce the length of recall of reproductive events). At least one member of the couple had to be working on the farm. Three questionnaires were designed to collect relevant information from the farm operator, husband, and wife on demographic and lifestyle information; pesticides currently and historically used on the farm and around the home; medical history; and a complete reproductive history.

The women in the study were asked to recall all their pregnancies, starting with their first. For spontaneous abortions, the woman was asked how many weeks pregnant she was (based on the last menstrual period) at the time of the abortion. We calculated the estimated calendar month of conception by subtracting the gestational age at abortion or delivery from the delivery date. The outcome of interest in this analysis was self-reported spontaneous abortion of less than 20 weeks' gestation. We examined subgroups of spontaneous abortions of less than 12 weeks' and 12–19 weeks' gestation to provide an indirect estimate of risk by likely frequency of chromosomal anomaly, a more common cause in early abortions (*10*). Pregnancies occurring when the woman was not living on the study farm and thus had unknown exposure status were excluded, as were pregnancies for which the study husband was not likely the father.

We pooled pesticide exposure information from the farm operator (the person responsible for the day-to-day operations of the farm, if different from the husband or wife), husband, and wife to construct a history of monthly agricultural and residential pesticide use. For each pesticide reported, we identified the active ingredients and uses using a database of registered pesticide products in Canada. Where possible, we categorized the active ingredients into chemical families. We divided all pesticides reported into four major classes of use: herbicides, insecticides, fungicides, and miscellaneous others (including those that could not be classified). We identified the active ingredients and chemical families that were most frequently used on the farms in the study, as well as those most likely to have adverse reproductive effects according to the literature. This categorization produced 17 pesticide unit variables that we examined in this study (Table 1).

Because only couples living on the farm were eligible for the study, the exposure assessment in this analysis was intended to capture potential occupational (direct) and residential (indirect) exposures. Because

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indirect exposures were possible, we could not completely separate the exposure statuses of the men and women. Most pesticide applications were done by the husband, with only 20% of the wives reporting handling of farm pesticides. No other information was available to validate the exposure assessments; however, we used both open-ended and checklist questions to obtain as complete a recall as possible.

We merged reproductive and pesticide exposure history data to create pesticide unit variables for months preceding and during each pregnancy. Exposure to pesticides was analyzed for two windows: preconception, the 4-month period from 3 months before conception to the calendar month of conception (consistent with potential sperm-mediated effects); and postconception, the 3-month period from the first calendar month after conception to the end of the first trimester (consistent with a fetotoxic effect). Exposures that occurred after a pregnancy loss but within the period of interest (i.e., first trimester) were not considered in assessing exposure status. We also created pregnancy-specific variables for all other time-related factors (parental age, smoking, farm activities, and alcohol and caffeine intake).

Statistical Analysis

We calculated crude odds ratios (ORs) using logistic regression for each combination of pesticide unit, exposure window, and gestational age at abortion category. Because no strong confounders were evident in previous analyses of these data (*6*) and our sample size was limited, we did not estimate adjusted risks. Nonexposed pregnancies were those not exposed during the time window to the pesticide unit of interest.

To assess the importance of the timing of exposure to the risk of spontaneous abortion, we compared preconception exposures to postconception in a combined model where preconception exposures were coded 1 and postconception exposures were the referent. Pregnancies exposed to a pesticide unit in both windows were excluded from this analysis. Similarly, we used an indicator to distinguish early (< 12 weeks' gestation) and late (12–19 weeks' gestation) fetal age at abortion to identify the major target site for pesticide toxicity (embryo or fetus). In this latter model, which analyzed only spontaneous abortions, we used the 12–19 weeks' gestational age abortions as the referent group.

To explore statistical interactions between the various pesticide units and other risk factors for spontaneous abortion, we used the Classification and Regression Tree (CART) method. This method has been discussed in detail by Breiman and colleagues (*11*). The CART method has been applied in other disciplines, for example in diagnosing chest pain (*12*) and recently in epidemiologic studies (*13,14*).

CART is a nonparametric method used to construct a classification rule for predicting what class of an object or case is based on the values of its predictor variables. A tree is constructed by recursively partitioning a data set into increasingly homogeneous (measured by the distribution of the outcome variable) descendant subsets (*11*). Partitioning is conducted using a single covariate at a time and is represented by a node (branch) in the tree. The top node of the tree is called the root node. Those nodes that are not split are called terminal nodes or leaves.

Our search for interaction effects using CART involved all 17 pesticides variables analyzed separately for each level (use class, chemical family, and active ingredient), as well as 21 possible risk factors for spontaneous abortion (e.g., maternal and paternal age, education, smoking status, alcohol and caffeine consumption, and family income).

Our small sample size prevented subgroup analyses of early and late spontaneous abortions. CART analysis was conducted using a commercially developed software, AnswerTree 2.0 (*15*). The Gini criterion was applied in the selection of best splits. ORs and 95% confidence intervals (CI) were calculated for each node using SAS (*16*).

Table 1. The 17 pesticide unit variables created in the Ontario Farm Family Health Study.

Pesticide use class	Chemical family	Active ingredient
Herbicide		Dicamba
		Glyphosate
	Phenoxy acetic acid	4-[2,4-dichlorophenoxy] butyric acid (2,4-DB)
	(Phenoxy herbicides)	2,4-dichlorophenoxyacetic acid (2,4-D)
		[4-chloro-2-methylphenoxy] acetic acid (MCPA)
	Triazine	Atrazine
		Cyanazine
	Organophosphate	
	Thiocarbamate	
Insecticide		Carbaryl
	Organophosphate	
Fungicide		Captan
	Thiocarbamate	
	Triazine	
Miscellaneous		

Results

Approximately 2,000 farm couples participated in the study, contributing 3,936 pregnancies for analysis including 395 spontaneous abortions. All but five of the abortions were reported by the women as medically confirmed. Mean gestational age was 10 weeks, with 57% of the spontaneous abortions occurring before 12 weeks' gestation. The women participating in the study were involved to varying extents in working on the farm. Forty-eight percent assisted with the harvesting of crops, 21% milked cows, 20% helped to prepare the land for planting, and 3% applied crop herbicides. The wives were generally better educated than their husbands, with almost 40% having a college or university degree compared to 28% for the men. More farm men (22%) than women (16%) were current smokers. More than 70% of the farm women drank less than one alcoholic beverage per week, whereas about 43% of the men drank at least once per week.

Critical Exposure Window

Although many of the results shown in Table 2 are not statistically significant, preconception exposure to glyphosate, triazines, thiocarbamates, herbicides, fungicides, and miscellaneous pesticides moderately increased the risk for all spontaneous abortions (< 20 weeks). When the analysis was restricted to early abortions (< 12 weeks), increased risks were observed for preconception exposure to phenoxy acetic acid herbicides (OR = 1.5; 95% CI, 1.1–2.1) and two of its constituents, 2,4-dichlorophenoxyacetic acid (2,4-D) (OR = 1.3; 95% CI, 0.9–2.0) and 2,4-DB (OR = 1.4; 95% CI, 0.7–2.8), in addition to the triazine chemical family and herbicide class of pesticides. For late spontaneous abortions (12–19 weeks), preconception exposure to thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), glyphosate (OR = 1.7; 95% CI, 1.0–2.9), fungicides (OR = 1.4; 95% CI, 0.9–2.1), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) were associated with elevated risks.

Risk estimates for the postconception exposure window are listed in Table 3. The risks associated with the miscellaneous class of pesticides were elevated for both early and late spontaneous abortions. Other elevations in risk were observed only in the late abortions after exposure to 2,4-D (OR = 1.6; 95% CI, 0.9–2.7), dicamba (OR = 1.6; 95% CI, 0.8–3.2), glyphosate (OR = 1.4; 95% CI, 0.8–2.5), and the phenoxy acetic acid herbicides (OR = 1.3; 95% CI, 0.8–2.0).

For most pesticides examined, preconception exposure contributed more to the risk of a spontaneous abortion than exposures during the first trimester. This was especially true for early abortions, as measured by the elevated odds ratios observed when models were constructed with exposure window as the outcome (Table 4). Analyses that incorporated gestational age at abortion as the outcome variable generally produced higher risk estimates for early spontaneous abortions from preconception exposure (Table 5). Except for cyanazine, carbaryl, and organophosphates, postconception exposures had more effect on the risk of late abortions, as measured by odds ratios less than one.

Interaction among Risk Factors

Overall, in the tree-based analysis, maternal age was the strongest risk factor observed for spontaneous abortions of less than 20 weeks' gestation. Maternal age partitioned the study population with a cut-off of 35 years of age. A pregnant woman age 35 or older was 2.6 times more likely to have a spontaneous abortion than a younger woman (95% CI, 1.7–3.9).

Among older women, preconception exposure to carbaryl and 2,4-D determined further refinement of these subgroups (Figure 1). Women age 35 or older who were exposed to carbaryl had nearly a 4-fold increase in risk compared to women of the same age who were not exposed. Pregnancies of women less than 35 years of age (node 2) were not at increased risk of a spontaneous abortion if exposed to any of the active ingredients during the preconception window. Node 2 is called a terminal node (or leaf) because further splitting could not generate an odds ratio different from one. Node 5 is further split into nodes 6 and 7. Based on a comparison of nodes 6 and 7, a pregnant woman 35 years or older exposed to both carbaryl and 2,4-D was 27 times more likely to have a spontaneous abortion than a woman in the same age range who was exposed to carbaryl only.

When the analysis was conducted at the chemical family level, we detected interaction effects between maternal age and preconception exposure to several pesticide families (Figure 2). The results suggested that a pregnant women age 35 or older who is exposed to triazines during the preconception window had nearly three times the risk (OR = 2.7; 95% CI, 1.1–6.9) of a spontaneous abortion. Furthermore, from nodes 6 and 7, we observed that preconception exposure to phenoxy acetic acid herbicides in the older group of women more than doubled the risk (OR = 2.3; 95% CI, 0.6–8.6). At nodes 8 and 9, we observed a three-way interaction effect among maternal age, triazines, and thiocarbamates, indicating that a pregnant woman 35 years or older who was exposed to both triazines and thiocarbamates before conception had a nearly 8-fold increase in risk over those exposed to triazines only. No such interaction was observed for younger women.

We also observed interactions between pesticide use classes (data not shown). Exposure to both fungicides and herbicides before conception doubled the risk relative to that for a woman who was exposed only to fungicides (OR = 2.0; 95% CI, 1.1–3.5). Among the older group of pregnant women, exposure to fungicides doubled the risk of having a spontaneous abortion compared to those not exposed (OR = 2.4; 95% CI, 1.0–5.9). No increased risk was observed among the younger women.

Interactions with maternal age were also found among postconception exposures to pesticides. Among older women exposed to glyphosate, the risk was three times that for women of the same age who were not exposed to this active ingredient (OR = 3.2; 95% CI, 0.8–23.0). Pregnant women age 35 or older exposed during the first trimester to thiocarbamates were at increased risk of spontaneous abortion (OR = 2.4; 95% CI, 0.5–10.5). Younger women exposed to the same chemical family were not at increased risk of an abortion. Pregnant women 35 or older exposed during pregnancy to the miscellaneous class of pesticides were at increased risk (OR = 2.5; 95% CI, 0.9–6.7).

Table 2. Spontaneous abortion risk and preconception exposure to various pesticides.

	All gestational ages	< 12 weeks		12–19 weeks	
Pesticide unit	Crude OR (95% CI)	No. of exposed cases[a]	Crude OR (95% CI)	No. of exposed cases[a]	Crude OR (95% CI)
Pesticide active ingredient					
Atrazine	1.2 (0.9–1.7)	24	1.3 (0.8–2.0)	16	1.1 (0.7–1.9)
Captan	1.0 (0.5–1.8)	6	1.0 (0.4–2.1)	5	1.0 (0.4–2.6)
Carbaryl	1.2 (0.9–1.7)	24	1.2 (0.8–1.9)	17	1.2 (0.7–2.0)
Cyanazine	0.7 (0.3–1.7)	4	0.9 (0.3–2.4)	2	0.6 (0.1–2.3)
2,4-D	1.2 (0.8–1.6)	26	1.3 (0.9–2.0)	13	0.9 (0.5–1.6)
2,4-DB	0.8 (0.4–1.5)	10	1.4 (0.7–2.8)	0	0.1 (0.0–1.4)
Dicamba	1.0 (0.7–1.7)	11	1.0 (0.5–1.8)	9	1.1 (0.6–2.2)
Glyphosate	1.4 (1.0–2.1)	16	1.1 (0.7–1.9)	17	1.7 (1.0–2.9)
MCPA	0.8 (0.5–1.3)	17	1.1 (0.6–1.8)	7	0.6 (0.3–1.2)
Chemical families					
Phenoxy acetic acid	1.2 (0.9–1.5)	48	1.5 (1.1–2.1)	21	0.8 (0.5–1.9)
Triazine	1.3 (1.0–1.8)	35	1.4 (1.0–2.0)	22	1.1 (0.7–1.8)
Organophosphate	1.0 (0.7–1.4)	24	1.0 (0.6–1.6)	18	1.0 (0.6–1.7)
Thiocarbamate	1.5 (1.0–2.1)	16	1.1 (0.7–1.9)	18	1.8 (1.1–3.0)
Use classes					
Herbicide	1.3 (1.0–1.6)	78	1.4 (1.1–1.9)	51	1.1 (0.8–1.6)
Insecticide	1.1 (0.9–1.4)	68	1.2 (0.9–1.5)	49	1.1 (0.8–1.5)
Fungicide	1.4 (1.1–1.8)	36	1.3 (0.9–1.9)	28	1.4 (0.9–2.1)
Miscellaneous	1.5 (1.1–2.0)	25	1.3 (0.8–2.1)	21	1.5 (1.0–2.4)

[a]The total number of cases of spontaneous abortion is 395, with 226 and 169 early and late abortions, respectively.

Table 3. Spontaneous abortion risk and postconception exposure to various pesticides.

	All gestational ages	< 12 weeks		12–19 weeks	
Pesticide unit	Crude OR (95% CI)	No. of exposed cases[a]	Crude OR (95% CI)	No. of exposed cases[a]	Crude OR (95% CI)
Pesticide active ingredient					
Atrazine	0.8 (0.5–1.2)	10	0.7 (0.3–1.5)	8	0.8 (0.4–1.6)
Captan	0.6 (0.3–1.4)	2	0.3 (0.1–1.4)	4	0.9 (0.3–2.5)
Carbaryl	0.8 (0.5–1.2)	14	0.9 (0.5–1.6)	7	0.6 (0.3–1.3)
Cyanazine	0.1 (0.0–0.9)	1	0.2 (0.0–1.4)	0	0.1 (0.0–2.4)
2,4-D	1.0 (0.7–1.6)	9	0.6 (0.3–1.2)	16	1.6 (0.9–2.7)
2,4-DB	0.4 (0.2–1.1)	1	0.2 (0.0–1.2)	3	0.7 (0.2–2.3)
Dicamba	1.1 (0.7–1.9)	6	0.8 (0.3–1.7)	9	1.6 (0.8–3.2)
Glyphosate	1.1 (0.7–1.7)	10	0.8 (0.4–1.6)	12	1.4 (0.8–2.5)
MCPA	0.8 (0.5–1.3)	8	0.7 (0.3–1.4)	8	0.9 (0.4–1.8)
Chemical families					
Phenoxy acetic acid	0.9 (0.6–1.2)	16	0.6 (0.4–1.0)	23	1.3 (0.8–2.0)
Triazine	0.7 (0.4–1.1)	12	0.6 (0.4–1.1)	11	0.8 (0.4–1.5)
Organophosphate	0.6 (0.4–1.0)	10	0.5 (0.3–1.0)	12	0.9 (0.5–1.5)
Thiocarbamate	0.8 (0.5–1.3)	7	0.6 (0.3–1.3)	9	1.1 (0.5–2.2)
Use classes					
Herbicide	0.8 (0.7–1.1)	37	0.7 (0.5–1.0)	38	1.1 (0.7–1.5)
Insecticide	0.8 (0.6–1.1)	40	0.7 (0.5–1.1)	37	1.0 (0.8–1.4)
Fungicide	0.8 (0.5–1.1)	16	0.6 (0.4–1.0)	18	1.0 (0.6–1.6)
Miscellaneous	1.7 (1.2–2.3)	25	1.4 (0.9–2.2)	24	1.9 (1.2–3.0)

[a]The total number of cases of spontaneous abortion is 395, with 226 and 169 early and late abortions, respectively.

The odds ratio for younger women exposed to the same group of chemicals was 1.5 (95% CI, 1.1–2.2). Furthermore, the risk for pregnant women 35 or older exposed to both miscellaneous pesticides and fungicides was 4.3 (95% CI, 0.3–57.6).

Discussion

Our results suggest that the critical window of exposure for spontaneous abortions of less than 20 completed weeks of gestation is during the 4-month period from 3 months before conception up to and including the calendar month of conception. Preconception exposure to the pesticide active ingredients glyphosate, atrazine, carbaryl, and 2,4-D was associated with a 20–40% relative increase in risk; whereas postconception exposures to any of the pesticide units tested (except the miscellaneous class of pesticides) was not associated with an increased risk. Pesticides belonging to the triazine, thiocarbamate, or phenoxy acetic acid chemical families were also associated with moderately increased risks.

Analysis of early (< 12 weeks) and late (12–19 weeks) spontaneous abortions revealed differences between the timing of exposure and the target, represented by the gestational age at abortion. Preconception exposure to the triazine (atrazine) and phenoxy herbicides (2,4-D and 2,4-DB) was associated with increased risks of early but not late spontaneous abortion. The herbicide glyphosate was associated with increased risks of late abortion, regardless of when exposure occurred. Generally, pregnancies exposed to pesticides before conception resulted in early abortions, suggesting a paternally mediated mechanism. There was some indication, measured by our comparison modeling, that postconception exposures were more likely associated with late abortions.

This finding has important implications for our understanding of the mechanism by which chemical exposures may cause spontaneous abortions. Previous studies have already suggested the existence of etiologic differences between early and late spontaneous abortions (*10,17*). Most early abortions have gross chromosomal anomalies (*18*). Our findings of an association between preconception exposure and an early abortion may imply that for some pesticides, preconception exposures lead to gross chromosomal anomalies. On the other hand, our finding of an association between late abortions and postconception exposure may suggest that postconception exposure to specific pesticides tends to damage the fetus or fetus–placenta complex rather than cause chromosomal anomalies.

We also found strong evidence of interaction between maternal age and pesticide exposure on the risk of spontaneous abortion in both exposure windows. Most of the increased risks associated with pesticide exposure were observed in women age 35 or older. Similar to the findings of other studies (*19,20*), we observed that advanced maternal age was associated with an increased risk of spontaneous abortion (crude OR = 2.6; 95% CI, 1.7–3.9). Trisomic oocytes and a less efficient uterus have been identified as independent risks for older women (*21*). Maternal age may also be a surrogate measure for cumulative exposure to various pesticides, other unknown factors, or accumulated toxicity for either parent, because it is often highly correlated with paternal age.

Although several epidemiologic studies of the reproductive toxicity of pesticides have been conducted suggesting increased risks of fetal deaths, few have focused on specific pesticide products or chemical families (*22*). The phenoxy herbicides have been one of the most commonly studied groups of pesticides. Genetic *in vitro* toxicity testing on the phenoxy herbicide 2,4-D has reportedly been negative (*23*). Paternally mediated reproductive toxicity of a picloram and 2,4-D combination herbicide has been suggested in mice (*24*). Human studies have shown that this pesticide may damage sperm (*25*), increase the risk of spontaneous abortion in wives of older farmers (ages 31–35) (*26*), and be measured in seminal fluid of applicators (*27*).

The triazine pesticide atrazine has caused chromosomal damage in Chinese hamster ovary cells (*28*) and been associated with elevated rates of intrauterine growth retardation in communities with contaminated drinking waters (*29*). However, there is conflicting evidence as to whether atrazine is mutagenic in cultured human cell lines (*30–33*). Atrazine has had adverse reproductive effects in rats, including fetal losses (*34*). Cyanazine has shown some teratogenic effects in rats (*35*).

The genotoxicity of glyphosate has been positive in *in vitro* cultures of bovine (*36*) and human lymphocytes (*32*) and weakly mutagenic in a *Salmonella* assay (*37*). Carbaryl, a carbamate pesticide, has been associated with increased risks of childhood brain cancer (*38*)

Table 4. Comparison analysis of effects of pre- versus postconception exposure to pesticides on spontaneous abortion.[a]

	All gestational ages	< 12 weeks			12–19 weeks		
Pesticide unit	Crude OR[b] (95% CI)	Preconception exposed cases	Postconception exposed cases	Crude OR (95% CI)	Preconception exposed cases	Postconception exposed cases	Crude OR (95% CI)
Pesticide active ingredient							
Atrazine	1.6 (0.9–3.0)	23	9	1.7 (0.8–3.8)	15	7	1.4 (0.6–3.5)
Captan	2.2 (0.6–8.5)	5	1	4.0 (0.5–35.4)	3	2	1.1 (0.2–7.1)
Carbaryl	2.0 (1.0–4.0)	17	7	1.7 (0.7–4.4)	15	5	2.2 (0.8–6.1)
Cyanazine	5.6 (0.7–47.5)	4	1	3.6 (0.4–33.1)	2	0	4.4 (0.2–94.3)
2,4-D	1.1 (0.6–2.0)	22	5	2.9 (1.1–8.0)	11	14	0.5 (0.2–1.1)
2,4-DB	1.8 (0.6–6.2)	10	1	7.8 (1.0–62.3)	0	3	0.1 (0.0–2.0)
Dicamba	1.0 (0.4–1.9)	9	4	1.4 (0.4–4.7)	8	8	0.6 (0.2–1.6)
Glyphosate	1.6 (0.7–3.4)	12	6	1.7 (0.6–4.2)	10	5	1.5 (0.5–4.6)
MCPA	1.1 (0.6–2.3)	14	5	2.0 (0.7–5.7)	7	8	0.6 (0.2–1.7)
Chemical families							
Phenoxy acetic acid	1.3 (0.8–2.1)	41	9	3.1 (1.4–6.4)	19	21	0.6 (0.3–1.1)
Triazine	1.9 (1.0–3.2)	34	11	2.1 (1.0–4.4)	20	9	1.4 (0.7–3.2)
Organophosphate	2.2 (1.0–4.8)	17	3	3.8 (1.1–13.4)	12	6	1.3 (0.4–3.6)
Thiocarbamate	2.5 (1.1–5.8)	13	4	2.0 (0.8–5.0)	13	4	2.4 (0.8–7.6)
Use classes							
Herbicide	1.6 (1.1–2.4)	59	18	2.3 (1.3–3.9)	36	23	1.1 (0.7–1.8)
Insecticide	1.7 (1.1–2.9)	38	10	2.6 (1.3–5.2)	28	16	1.2 (0.6–2.2)
Fungicide	2.8 (1.4–5.4)	25	5	3.9 (1.4–10.3)	17	7	1.8 (0.7–4.4)
Miscellaneous	0.6 (0.3–1.4)	5	5	0.8 (0.2–3.0)	5	8	0.5 (0.2–1.6)

Pregnancies with both pre- and postconception exposure have been excluded from the analysis in this table.
[a]Postconception exposure window used as referent group. [b]The odds ratios estimate the risk that exposures to pesticides resulting in a spontaneous abortion occurred in the preconception window, relative to the postconception window.

and reproductive and developmental effects in animals (*39*). Captan may be a potential clastogenic agent (*40*).

There is evidence that organophosphate pesticides have genotoxic effects in humans (*41*). Workers in Chinese pesticide factories exposed to organophosphate pesticides had moderately increased prevalences of sperm aneuploidy (*42*). Methamidophos, an organophosphate, may have the potential to affect male fertility and to produce transmissible adverse embryonic effects after an acute paternal germline exposure (*43*).

Although this study is one of the first to collect and analyze detailed information on the timing and types of pesticides used on farms and reproductive outcomes, several limitations suggest that our findings be interpreted with caution. Because dose information was not available, misclassification of exposure is likely. Many factors including the pesticide formulation, application conditions, handling practices, and interindividual differences in absorption, distribution, metabolism, and excretion of the products or metabolites will lead to variability in the degree of exposure. Because the farmers used many different pesticides during the study and our sample size was limited, findings may be unreliable, particularly for multiple pesticide interactions. Because pesticide products were reported primarily by the farm applicator or husband, differential recall of pesticide exposure by the mother is not likely to be a problem in this study; however, some nondifferential recall of pesticides and spontaneous abortions is likely. Because the analyses were designed to generate, not to test, hypotheses, and multiple comparisons were conducted, results should be interpreted with care and tested in other studies.

Also worthy of consideration is the fact that couples contributed multiple pregnancies to the analyses, and pregnancies from the same woman are not independent events. In previous analyses (*6*, *7*), generalized estimating equation models were constructed to account for this nonindependence and were found to have a modest effect on the confidence interval with little consequence on the effect measure. We also did not control for history of prior spontaneous abortion because these losses might have been caused partly by pesticide exposure and resulted in biased risk estimates (*44*). However, poor outcomes in previous pregnancies might alter behavior in subsequent pregnancies; for example, the woman might be more careful to avoid exposures to perceived toxic agents after experiencing a spontaneous abortion. Because this study has no personal pesticide dose information for either parent, we cannot rule out this potentially modifying effect. All the exposure information pertained to certain pesticides that were reported by either the farm operator or couple (mostly by the farm operator) as being used on the farm during a particular calendar period. We did not have information on the specific dates that each pesticide was applied, nor did we expect that the farm operators would be able to report these dates accurately. Consequently, depending on when during the calendar month conception occurred, exposure during the estimated month of conception may have been incorrectly assigned to the preconception window. In an earlier article (*6*) we looked at variations in the time window of interest. The pattern of risks during the estimated calendar month of conception for spontaneous abortions following phenoxy herbicide exposures was similar to that seen for the preconception window. Accordingly, the

Table 5. Odds of early versus late spontaneous abortion after exposure to pesticides at different times.[a]

	Preconception exposure			Postconception exposure		
Pesticide unit	No. cases < 12 weeks	No. cases 12–19 weeks	Crude OR[b] (95% CI)	No. cases < 12 weeks	No. cases 12–19 weeks	Crude OR (95% CI)
Active ingredient						
Atrazine	24	16	1.1 (0.6–2.2)	10	8	0.9 (0.4–2.4)
Captan	6	5	0.9 (0.3–2.9)	2	4	0.4 (0.1–2.0)
Carbaryl	24	17	1.1 (0.6–2.1)	14	7	1.5 (0.6–3.8)
Cyanazine	4	2	1.5 (0.3–8.3)	1	0	2.2 (0.1–55.6)
2,4-D	26	13	1.6 (0.8–3.1)	9	16	0.4 (0.2–0.9)
2,4-DB	10	0	16.4 (0.9–282.0)	1	3	0.2 (0.0–2.4)
Dicamba	11	9	0.9 (0.4–2.2)	6	9	0.5 (0.2–1.4)
Glyphosate	16	17	0.7 (0.3–1.4)	10	12	0.6 (0.2–1.4)
MCPA	17	7	1.8 (0.8–4.6)	8	8	0.7 (0.3–2.0)
Chemical families						
Phenoxy acetic acid	48	21	1.9 (1.1–3.3)	16	23	0.5 (0.2–0.9)
Triazine	35	22	1.2 (0.7–2.2)	12	11	0.8 (0.3–1.9)
Organophosphate	24	18	1.0 (0.5–1.9)	10	12	1.6 (0.3–1.4)
Thiocarbamate	16	18	0.6 (0.3–1.3)	7	9	0.6 (0.2–1.6)
Use classes						
Herbicide	78	51	1.2 (0.8–1.9)	37	38	0.7 (0.4–1.1)
Insecticide	68	49	1.1 (0.7–1.6)	40	37	0.8 (0.5–1.3)
Fungicide	36	28	1.0 (0.6–1.6)	16	18	0.6 (0.3–1.3)
Miscellaneous	25	21	0.9 (0.5–1.6)	25	24	0.8 (0.4–1.4)

[a]Only spontaneous abortions were analyzed, with the 12–19 weeks' gestational age category used as referent group. [b]The odds ratios estimate the risk that an early abortion, relative to a later abortion, occurred in either of the two exposure windows.

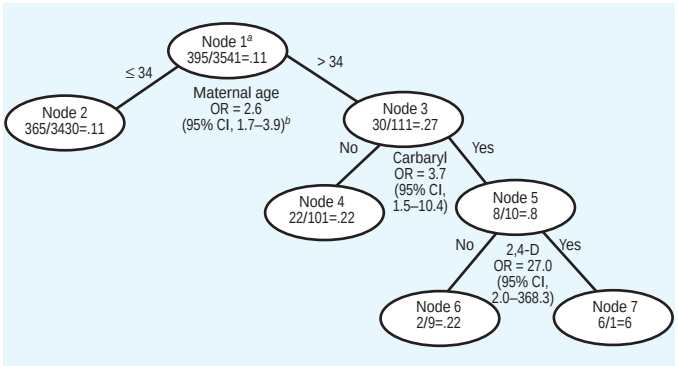

Figure 1. Classification and Regression Tree analysis of crude spontaneous abortion risk (< 20 weeks' gestation)—preconception exposure to pesticide active ingredients and other risk factors.
[a]In the nodes, the numerators represent the number of cases; the denominators are the number of non-cases. [b]Left branch of node used as referent group.

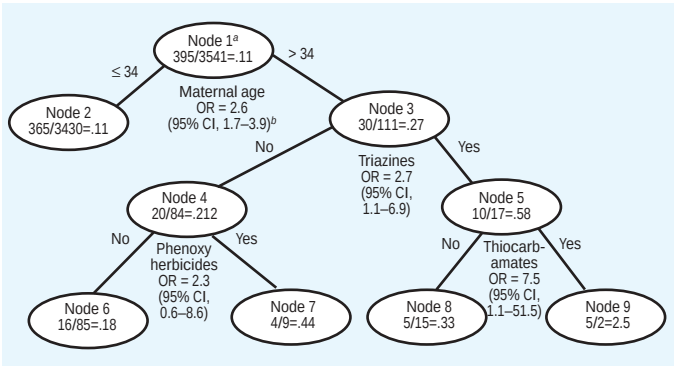

Figure 2. Classification and Regression Tree analysis of crude spontaneous abortion risk (< 20 weeks' gestation)—preconception exposure to pesticide chemical families and other risk factors.
[a]In the nodes, the numerators represent the number of cases; the denominators are the number of non-cases. [b]Left branch of node used as referent group.

calendar month of conception was included in the preconception window in the current analyses.

Our analyses did not consider the half-lives of the individual pesticides. Several of the herbicides, such as those in the phenoxy family, have relatively short half-lives, whereas others may have longer half-lives or persist in the environment. In addition, we examined only the active ingredients, not the so-called inert ingredients in pesticide products. Some of the inert ingredients may contribute to the potential toxicity of the pesticide product. Unfortunately, much of this information is not readily available.

The referent group in most of the analyses reported here (Tables 2 and 3) comprised pregnancies not exposed to the pesticide of interest during the window under consideration. In an earlier article (*6*) in which the referent group was pregnancies not exposed to any pesticides during the window, we reported a crude odds ratio for early abortions of 2.3 (95% CI, 1.0–5.6) for preconception exposure to phenoxy herbicides. Here we report an odds ratio of 1.5 (95% CI, 1.1–2.1), showing the attenuation in risk when a different referent group is used.

Exploring statistical interaction between pesticides and other risk factors is one of the contributions of this article to the literature. Previous studies have lacked sufficient detail on pesticide products to allow for such a comparison. The statistical techniques most commonly used to assess statistical interaction and to control for confounders are logistic regression and stratified analysis (*45*). However, these two methods are designed primarily for hypothesis or theory testing with few predictor variables. In an exploratory study of statistical interaction, both methods are extremely time consuming when the number of combinations of two-way or three-way interactions is large (*46*).

The CART method has several advantages over traditional methods in an exploratory study, especially with a large data set (*12,14,47*). It helps researchers identify important predictor variables and cut points for continuous variables. It can also detect various linear and nonlinear statistical interactions through defining higher-risk subpopulations. Nevertheless, the use of CART also has some caveats. One of the problems is that the same predictor variable may be selected to split a number of successive levels. As a result, there is a tendency to select predictor variables that can afford more splits in the tree-growing process. To overcome this problem, we used a user-controlled tree-growing approach. With this approach, we determined the priority of predictor variable selection based on the value of the Gini criterion; at the same time, we avoided repeat selections of the same predictor variable in different levels of a tree. This process allows for growing a tree with a reasonable number of levels or branches. The statistical findings based on an overgrown tree with repeat selection of the same variable are often associated with problems of low reliability, where a small alteration in the number of cases may lead to statistically significant changes in risk estimates (*48*).

Historically, research has focused on the critical periods of human development and the ways the effect produced by a given agent might be expected to change when exposure occurs at different times during pregnancy. More recently, evidence shows that critical windows of exposure also encompass preconceptional and postnatal (neonatal, peripubertal/adolescent) time periods (*49*). Our contrasting results for early versus late spontaneous abortions suggest that the developing organism is differentially sensitive to various pesticides at critical periods of development. During the preconception window, damage to spermatozoal DNA can be transmitted to the zygote and may cause early embryo death (*50*). Because the herbicide 2,4-D has been measured in seminal fluid (*27*), there is evidence that at least some pesticides may be delivered to the target site where damage to the spermatozoa could occur.

Although this study was not able to provide any information on dose, it did show that timing of exposure may be as important as dose in characterizing the reproductive toxicity of a chemical product (*4*). Identifying the windows in time when the reproductive system is most sensitive will provide insight into the underlying pathology. Given our results, we recommend that future studies employ a similar statistical methodology to identify potentially toxic agents and mixtures, and examine closely the role of advanced maternal age in the degree of toxicity of an agent. Further epidemiologic research on the reproductive toxicity of glyphosate, carbaryl, the phenoxy acetic acid and triazine herbicides, and thiocarbamate pesticides is warranted.

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