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), thiacarbamates (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, thiacarbamates, triazine, windows of vulnerability. Environ Health Perspect 109:851–857 (2001). [Online 14 August 2001] http://ehpnet1.niehs.nih.gov/docs/2001/109p851-857arbuckle/abstract.html

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 woman 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.

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 sources 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.
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 subsets. The CART method has been applied in other disciplines, for example in diagnosing chest pain (12) and recently in epidemiologic studies (13,14).

Our search for interaction effects using CART involved all 17 pesticide 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).

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, 0.8–2.8), dicamba (OR = 1.6; 95% CI, 0.8–2.8), and the phenoxy acetic acid herbicides (OR = 1.3; 95% CI, 0.8–2.0).

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

| Pesticide use class | Chemical family | Active ingredient |
|---------------------|-----------------|-------------------|
| Herbicide           |                 |                   |
| Phenoxy acetic acid |                 |                   |
| Triazine            |                 |                   |
| Organo phos phate   |                 |                   |
| Thio carbamate      |                 |                   |
| Insecticide         |                 |                   |
| Fungicide           |                 |                   |
| Miscellaneous       |                 |                   |

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**Table 2.** Odds ratios (OR) and 95% confidence intervals (CI) for spontaneous abortions by pesticide unit exposure for the first trimester. The ORs and 95% CI were calculated for each pesticide unit using AnswerTree 2.0. The Gini criterion was applied in the selection of best splits. ORs and 95% confidence intervals (CI) were calculated for each pesticide unit using AnswerTree 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. A 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 it was not at increased risk of a spontaneous abortion. 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 woman 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 acid herbicides in the older group of women more than doubled the risk (OR = 2.3; 95% CI, 0.6–6.8). At nodes 8 and 9, we observed a three-way interaction effect among maternal age, triazines, and thiocarbamates, indicating that a pregnant woman age 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).

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

| Pesticide active ingredient | <12 weeks (Crude OR (95% CI)) | No. of exposed cases | >12 weeks (Crude OR (95% CI)) | No. of exposed cases | <12 weeks (Crude OR (95% CI)) | No. of exposed cases |
|----------------------------|--------------------------------|---------------------|--------------------------------|---------------------|--------------------------------|---------------------|
| Atrazine                   | 1.2 (0.9–1.7)                  | 24                  | 1.3 (0.8–2.0)                  | 16                  | 1.1 (0.7–1.9)                  | 16                  |
| Captan                     | 1.0 (0.5–1.8)                  | 6                   | 1.0 (0.4–2.1)                  | 5                   | 1.0 (0.4–2.6)                  | 5                   |
| Carbaryl                   | 1.2 (0.9–1.7)                  | 24                  | 1.2 (0.8–1.9)                  | 17                  | 1.2 (0.7–2.0)                  | 17                  |
| Cyanazine                  | 0.7 (0.3–1.7)                  | 4                   | 0.9 (0.3–2.4)                  | 2                   | 0.6 (0.1–2.3)                  | 2                   |
| 2,4-D                      | 1.2 (0.8–1.6)                  | 26                  | 1.3 (0.9–2.0)                  | 13                  | 0.9 (0.5–1.6)                  | 13                  |
| 2,4-DI                     | 0.8 (0.4–1.5)                  | 10                  | 1.4 (0.7–2.8)                  | 0                   | 0.1 (0.0–1.4)                  | 0                   |
| Dicamba                    | 1.0 (0.7–1.7)                  | 11                  | 1.0 (0.5–1.8)                  | 9                   | 1.1 (0.6–2.2)                  | 9                   |
| Glyphosate                 | 1.4 (1.0–2.1)                  | 16                  | 1.1 (0.7–1.9)                  | 17                  | 1.7 (1.0–2.9)                  | 17                  |
| M CPA                      | 0.8 (0.5–1.3)                  | 17                  | 1.1 (0.6–1.8)                  | 7                   | 0.6 (0.3–1.2)                  | 7                   |
| 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)                  | 21                  |
| Thiazole                   | 1.3 (1.0–1.8)                  | 35                  | 1.4 (1.0–2.0)                  | 22                  | 1.1 (0.7–1.8)                  | 22                  |
| Organophosphate            | 1.0 (0.7–1.4)                  | 24                  | 1.0 (0.6–1.6)                  | 18                  | 1.0 (0.6–1.7)                  | 18                  |
| Thiolecarbamate            | 1.5 (1.0–2.1)                  | 16                  | 1.1 (0.7–1.9)                  | 18                  | 1.8 (1.1–3.0)                  | 18                  |
| Use classes                |                                |                     |                                |                     |                                |                     |
| Herbicide                  | 1.3 (1.0–1.6)                  | 78                  | 1.4 (1.1–1.9)                  | 51                  | 1.1 (0.8–1.6)                  | 51                  |
| Insecticide                | 1.1 (0.9–1.4)                  | 68                  | 1.2 (0.9–1.5)                  | 49                  | 1.1 (0.8–1.5)                  | 49                  |
| Fungicide                  | 1.4 (1.1–1.8)                  | 36                  | 1.3 (0.9–1.9)                  | 28                  | 1.4 (0.9–2.1)                  | 28                  |
| Miscellaneous              | 1.5 (1.2–2.0)                  | 25                  | 1.3 (0.8–2.1)                  | 21                  | 1.5 (1.0–2.4)                  | 21                  |

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

| Pesticide unit | All gestational ages | 12-19 weeks | <12 weeks | 12-19 weeks |
|----------------|---------------------|-------------|-----------|-------------|
|                | Crude OR (95% CI)   | No. of exposed cases | Crude OR (95% CI) | No. of exposed cases |
| Pesticide active ingredient |                                |                     |                                |                     |
| Atrazine       | 0.8 (0.5–1.3)       | 10           | 0.7 (0.3–1.5) | 8           | 0.8 (0.4–1.6) | 8 |
| Captan         | 0.6 (0.3–1.2)       | 2            | 0.3 (0.1–1.4) | 4           | 0.9 (0.3–2.5) | 4 |
| Carbaryl       | 0.8 (0.5–1.2)       | 14           | 0.9 (0.5–1.6) | 7           | 0.6 (0.3–1.3) | 7 |
| Cyanazine      | 0.1 (0.0–0.9)       | 1            | 0.2 (0.0–1.4) | 0           | 0.1 (0.0–2.4) | 0 |
| 2,4-D          | 1.0 (0.7–1.6)       | 9            | 0.6 (0.3–1.2) | 16          | 1.6 (0.9–2.7) | 16 |
| 2,4-DI         | 0.4 (0.2–1.1)       | 1            | 0.2 (0.0–1.2) | 3           | 0.7 (0.2–2.3) | 3 |
| Dicamba        | 1.1 (0.7–1.9)       | 6            | 0.8 (0.3–1.7) | 9           | 1.6 (0.8–3.2) | 9 |
| Glyphosate     | 1.1 (0.7–1.7)       | 10           | 0.8 (0.4–1.6) | 12          | 1.4 (0.8–2.5) | 12 |
| M CPA          | 0.8 (0.5–1.3)       | 8            | 0.7 (0.3–1.4) | 8           | 0.9 (0.4–1.8) | 8 |

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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 cows 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 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

| Pesticide unit | All gestational ages | 12–19 weeks |
|---------------|---------------------|-------------|
|               | Preconception       | Postconception | Preconception | Postconception | Preconception | Postconception |
|               | Crude OR (95% CI)   | Crude OR (95% CI) | Crude OR (95% CI) | Crude OR (95% CI) | Crude OR (95% CI) | Crude OR (95% CI) |

Pregnancies with both pre- and postconception exposure have been excluded from the analysis in this table. aPostconception exposure window used as referent group. bThe 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 organophosphorus, may have the potential to affect male fertility and to produce transmissible adverse embryonic effects after an acute paternal germine 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 pertinent 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 might 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 is 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.](image)

| Pesticide unit | Preconception exposure | Postconception exposure |
|----------------|------------------------|-------------------------|
|                | No. cases | Cases | Crude ORa (95% CI) | No. cases | Cases | Crude ORa (95% CI) |
| Active ingredient |          |       |                   |          |       |                   |
| Atrazine       | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Captan         | 35/30      | 20/60 | 1.0 (0.8–1.3)     | 35/30     | 20/60 | 1.0 (0.8–1.3)     |
| Carbaryl       | 40/30      | 20/60 | 1.0 (0.8–1.3)     | 40/30     | 20/60 | 1.0 (0.8–1.3)     |
| Cyanyazine     | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| 2,4-D          | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| 2,4-DB         | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Dicamba        | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Glyphosate     | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| MCPA           | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Chemical families |         |       |                   |          |       |                   |
| Phenoxy acetic acid | 10/3 | 20/60 | 1.0 (0.8–1.3) | 10/30 | 20/60 | 1.0 (0.8–1.3) |
| Triazine       | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Organophosphate | 10/3 | 20/60 | 1.0 (0.8–1.3) | 10/30 | 20/60 | 1.0 (0.8–1.3) |
| Thiocarbamate  | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Use classes |              |       |                   |          |       |                   |
| Herbicide      | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Insecticide    | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Fungicide      | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |
| Miscellaneous  | 10/3        | 20/60 | 1.0 (0.8–1.3)     | 10/30     | 20/60 | 1.0 (0.8–1.3)     |

aOnly spontaneous abortions were analyzed, with the 12-19 weeks’ gestational age category used as referent group.
bThe 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.](image)

![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.](image)
calendrical 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- 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 warrants.

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