Maternal self-reported exposure to pesticides in a Polish rural population and its effect on birthweight – example of cross-sectional study
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Aim of the exercise:

- To use an epidemiological approach to examine the effect of exposure of pregnant women to pesticides on the birthweight of the infants.
- To explain step by step, the elements of cross-sectional study (i.e. the selection of study population, methods preparation, results recording, analysis of results and their interpretation).
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Introduction (1)

*In which situations pregnant women living and working in rural areas might be exposed to pesticides?*

- The women living on farms are rarely involved, especially during pregnancy, in the mixing of chemicals, loading and cleanup of equipment and disposal of empty containers.

- However, they may be present on the field during the application of pesticides, take care of the clothing worn by the applicators and be subject to re-entry exposure while entering the field after spraying.
Have there been reports that farming activities might affect the birthweight? Do we really need an epidemiological study to address this problem?

- Farmers in general, compared to non-farmers, have more favourable birth characteristics including lower rates of small-for-gestational age infants (SGA), which may obscure the potential risk factors inherent in farmers’ jobs.
- There were few epidemiological studies indicating that maternal exposure to pesticides may contribute to SGA births.
- However different scenarios of exposure in different countries or even country region did not allow for definite statements.
Which other factors affecting birthweight should be taken into account?

- It is well known that in the absence of chronic maternal and foetal disease, birth weight is affected by the duration of pregnancy, sex of the infant, socio-economic variables (education, marital status) and intrauterine exposure to tobacco smoke.
• The goal of the present study was to evaluate the impact of maternal exposure to pesticides on birth weight in a population of farmers in rural Poland.
The study population included women living in Zadzim, Poddębice County, Central Poland, who had delivered at least one child between January 1, 1994 and December 31, 2000.

Identification of all women (N=123) who met this criteria was provided by the County Maternity Unit.
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Methods (2)- recruitment of subjects

- Ten women identified were excluded due to chronic health problems during pregnancy (diabetes, hypertension, heart or kidney problems).

- Of the remaining 113 women, 104 (92%) participated, six women refused and three were permanently away from home or unable to be reached/or contacted.
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Methods (3) - questionnaires

Questionnaire was administered by a midwife to all recruited subjects.

A questionnaire included:

- mother’s demographic
- anthropometric characteristics (weight and height),
- job history,
- smoking
- reproductive history (spontaneous and elective terminations, stillbirths and live births, including number, duration, birth weight and sex of each infant)
- type of farming (crops, orchards, around home activities)
- mother’s and other family members’ involvement in field labor,
- other non-farming jobs
- trade names of pesticides used within the last six years (open-ended questions).
**Methods (4) – exposure assessment**

*Information on the trade names of pesticide(s) used*

Each mother was asked to:

- verify the trade name and the timing of the pesticide(s) used, with the person directly involved in the application (most often her husband).
- describe her personal involvement (applicator, observer, not involved) in each pesticide applications session.
- provide history of pesticide use on the farm during the three months’ immediately preceding conception period and in three trimesters of pregnancy.

Based on the trade names of pesticides reported, the names of active ingredients were identified using a database of registered pesticides in Poland and classified into chemical groups.
To test for differences between subgroups the T test was used.

In order to determine whether the birthweight differed between infants of mothers exposed and unexposed to pesticides multiple linear regression model was built by SPSS (Statistical Package for Social Science) software.

The model included pregnancy duration, exposure to pesticides as well as other variables known to affect birthweight (infant sex, maternal pre-pregnancy weight, height, smoking during pregnancy, calendar year of birth, involvement in field work).
Most frequent among the examined population were mothers engaged in crop farming (50%), followed by those involved mostly in non-farming jobs (32.7%) and lastly orchards owners (17.3%).

Farms reported were usually family run and were approximately 50 000 – 100 000 m² in size.
Results(2) - use of pesticides

What information about the use of pesticides during pregnancy you can derive from table below?

<table>
<thead>
<tr>
<th>Type of farming</th>
<th>Mostly non-farming jobs (n=34)</th>
<th>Orchards (n=18)</th>
<th>Crop farming (n=52)</th>
<th>All examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Not used</td>
<td>34</td>
<td>100</td>
<td>3</td>
<td>16.7</td>
</tr>
<tr>
<td>Used in 1(^{st}) or 2(^{nd}) trimester</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>83.3</td>
</tr>
</tbody>
</table>

- Pesticide use during the first or second trimester of pregnancy was reported by 73% of mothers working on crop farms and 83.3% of those working in orchards.
- Across all groups, the timing of exposure tended to occur more often in the first rather than the second trimester.
Results (3) – pregnant women involvement in field work during pregnancy?
Women involved in what type of farming were more often involved in field work during pregnancy?

<table>
<thead>
<tr>
<th>Work in field during pregnancy</th>
<th>Mostly non-farming jobs (n=34)</th>
<th>Type of farming</th>
<th>All examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Did not work in field (n=31)</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Worked in field (n=22)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

- Among mothers working in orchards, 44.4% reported involvement in field work compared to 26.9% engaged in crop farming.
### Results (4) - use of pesticides

**What type of pesticides women reported to use?**

**Were there differences by type of farming?**

<table>
<thead>
<tr>
<th>Chemical groups of pesticide</th>
<th>Type</th>
<th>Orchards (n=15)</th>
<th>Crop farming (n=38)</th>
<th>Total (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Phenoxyacetic acid derivatives</td>
<td>H</td>
<td>9</td>
<td>60.0</td>
<td>21</td>
</tr>
<tr>
<td>Synthetic pyrethroids</td>
<td>I, A</td>
<td>9</td>
<td>60.0</td>
<td>17</td>
</tr>
<tr>
<td>Benzenothiosulfonate derivatives</td>
<td>I</td>
<td>2</td>
<td>13.3</td>
<td>15</td>
</tr>
<tr>
<td>Organophosphorous compounds</td>
<td>I, A</td>
<td>6</td>
<td>40.0</td>
<td>8</td>
</tr>
</tbody>
</table>

*H* - herbicides; *I* –insecticides; *A* - acaricides
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Results (5) - use of specific pesticides

- Phenoxyacetic acid derivatives were the most frequently reported type of pesticide used (58.8%), followed by synthetic pyrethroids (51%), benzensulfonothioate derivatives (33.3%) and organophosphorous compounds (27.5%)
- Except for benzenothiosulfonate derivatives, all of the above mentioned pesticides were used more often in orchards than in crop farming.
- Exposure to other types of compounds was much less prevalent in each group.
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Results (6) - use of the pesticides and pregnancy outcome

How would you describe the differences in pregnancy outcome (birthweight and pregnancy duration of women involved in different types of farming?)

<table>
<thead>
<tr>
<th>Type of farming</th>
<th>Mostly non-farming jobs (N=51)</th>
<th>Orchards (N=15)</th>
<th>Crop farming (N=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight (SD)</td>
<td>3409(517)</td>
<td>3597(325)</td>
<td>3294(461)</td>
</tr>
<tr>
<td>Av. Pregnancy duration (weeks)</td>
<td>39.4(1.2)</td>
<td>39.4(1.1)</td>
<td>39.1(1.2)</td>
</tr>
</tbody>
</table>
Results (7) – socio-economic characteristics of women involved in different types of farming

Have the women involved in different types of farming in such characteristics as weight, height and smoking?

<table>
<thead>
<tr>
<th>Type of farming</th>
<th>Mostly non-farming jobs (N=51)</th>
<th>Orchards (N=15)</th>
<th>Crop farming (N=38)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3294(461)</td>
</tr>
<tr>
<td>Av. Pregnancy duration (weeks)(SD)</td>
<td>39.4(1.2)</td>
<td>39.4(1.1)</td>
<td>39.1(1.2)</td>
</tr>
</tbody>
</table>
The mean birth weight of infants delivered by mothers working in crop farming was slightly lower compared to those with non-farming jobs.

In contrast, mothers working in orchards delivered infants with a slightly higher birth weight than non-farming women did.

The average pregnancy duration was similar in all the examined groups.

Mothers in each group were also similar with respect to other factors affecting birth weight, such as maternal weight and height.

The observed differences in birth weight could not be related to cigarette smoking, as mothers who reported either crop farming or work in orchards were characterized by a very low prevalence of smoking.
Have there been differences in pregnancy outcome by type of involvement in field work?

If so how would you evaluate the statistical significance of them?

<table>
<thead>
<tr>
<th>Field work involvement</th>
<th>Did not work (n=31)</th>
<th>Worked (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight (SD)</td>
<td>3243(452)*</td>
<td>3573(365)*</td>
</tr>
<tr>
<td>Av. Pregnancy duration (weeks)(SD)</td>
<td>39.2(1.3)</td>
<td>39.2(1.0)</td>
</tr>
</tbody>
</table>

*p=0.006*
Mothers who reported involvement in fieldwork had a similar pregnancy duration but delivered infants with a significantly higher birth weight than mothers not reporting such activities.
Have there been differences in pregnancy outcome by type of involvement in field work?

If so how would you evaluate the statistical significance of them?

<table>
<thead>
<tr>
<th>Field work involvement</th>
<th>Did not work (n=31)</th>
<th>Worked (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. Maternal weight (SD)</td>
<td>61.8(10.5)</td>
<td>64.0(11.0)</td>
</tr>
<tr>
<td>Av. Maternal height (SD)</td>
<td>162.5(7.9)</td>
<td>162.4(5.7)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>2(6.5)</td>
<td>1(4.6)</td>
</tr>
</tbody>
</table>
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Results (12) socio-economic characteristics by women involvement in the work in the field

- Both groups had a rather low proportion of smokers and similar height
- Women reporting field work had a slightly higher body weight.
Results (13) – use of pesticides and pregnancy outcome

Which group you would propose to compare to evaluate the effect of pesticides use on pregnancy outcomes?

How would you describe the differences in pregnancy outcomes by pesticide use?

<table>
<thead>
<tr>
<th>Pesticide use</th>
<th>Not used (N=51)</th>
<th>In 1st or 2nd trimester (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birth weight (SD)</td>
<td>3409 (461)</td>
<td>3380 (446)</td>
</tr>
<tr>
<td>Av. pregnancy duration (weeks)(SD)</td>
<td>39.4(1.2)</td>
<td>39.2(1.1)</td>
</tr>
</tbody>
</table>
Information about which women’s individual characteristics affecting pregnancy outcome we should have information?

<table>
<thead>
<tr>
<th></th>
<th>Pesticide use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not used (N=51)</td>
</tr>
<tr>
<td>Av. maternal weight (SD)</td>
<td>61.9(9.8)</td>
</tr>
<tr>
<td>Av. maternal height (SD)</td>
<td>163.7(5.6)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>9(17.7)</td>
</tr>
<tr>
<td>Field work</td>
<td>0</td>
</tr>
</tbody>
</table>
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Results (14) - birth weight and pesticide exposure

- Children of women living on farms where no pesticides were used during the first or second trimesters of pregnancy, despite the higher proportion of smokers, had slightly higher birth weight.

- Pregnancy duration and anthropometric characteristics of mothers were similar in both groups.
The groups of women compared with regard to pesticide use in the first and second trimesters of pregnancy differed in respect to potential confounding variables.

What type of statistical analysis you would propose to overcome this problem?
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### Results (16) - *Linear regression model*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coeff.</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxyacetic acid derivatives</td>
<td>-107.8</td>
<td>0.229</td>
</tr>
<tr>
<td>Synthetic pyrethroids</td>
<td>-196.0</td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>Benzenothiosulfonate derivatives</td>
<td>141.2</td>
<td>0.216</td>
</tr>
<tr>
<td>Organophosphorous compounds</td>
<td>123.3</td>
<td>0.301</td>
</tr>
<tr>
<td>Other pesticides</td>
<td>-12.1</td>
<td>0.894</td>
</tr>
<tr>
<td>Prepregnancy maternal weight</td>
<td>12.4</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>-139.3</td>
<td>0.246</td>
</tr>
<tr>
<td>Sex of infant</td>
<td>38.2</td>
<td>0.625</td>
</tr>
<tr>
<td>Field work</td>
<td>304.8</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Year of birth *</td>
<td>-207.8</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>Pregnancy duration</td>
<td>174.2</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Constant</td>
<td>-3960.03</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Year of birth was included to allow for time trends in birth weight, and woman’s direct involvement in field work.*
Results (17) How would you describe the results received?

- The exposure to synthetic pyrethroids pesticides was found to be an independent factor negatively associated with birth weight.
- Other factors identified as significant included: pregnancy duration, maternal prepregnancy weight, year of birth and involvement in field work.
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Discussion (1)- What is the main limitation of the presented study

The major limitation was assessment of pesticide exposure based on questionnaires.

However,

- several steps to obtain valid assessments were undertaken. The source of information about pesticide exposure was the person directly involved in farming and he was encouraged to recall the details of exposure using leaflets from the pesticide manufacturers and labels from used containers.

- The pattern of pesticide use was very much the same every year, so even the information referring to relatively remote events could be treated as highly reliable.
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Discussion (2) - how valid is the use of questionnaire for exposure assessment?

• The results of the National Cancer Institute studies indicate that the data obtained from standardized questionnaires may be reasonable indicators of occupational exposure when biomarker data are not available.

• The farmer carefully selects the pesticide most likely to be effective, purchases the pesticide, records the purchase (costs are tax deductible), mixes and applies the pesticide and evaluates the effectiveness of treatment. All these activities tend to reinforce their memory.

• All together, the validity of retrospective exposure assessment to pesticides, in spite of some limitations, is quite reassuring.
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Discussion (3) -should the biologic monitoring methods be used?

- Although the methods of biological monitoring seem to be the optimal way of assessing individual exposure to pesticides, such an approach is possible only in the case of cross-sectional (hospital deliveries) and prospective cohort studies.

- These methods have not as yet been introduced to retrospective assessment but have been strongly recommended.

- However, it has been recently stated that "biological monitoring may not be suitable when the nature of the work makes it difficult to pinpoint a priori which exposures among a broad range of possible contaminants are more likely than others".
The present study revealed a small but statistically significant effect of self-reported maternal pesticide exposure during the first or second trimester of pregnancy trimester on birthweight.

This was found after adjustment for pregnancy duration and the confounders including smoking and physical work during pregnancy.

Are results received consistent with other studies?
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Discussion (5) - results of studies in agriculture or farming population

Only a few reports addressed the risk of SGA in farmers’ infants. Two of them related the risk to the occupation labeled as “agriculture” or “farmer”.

- In Scotland, an analysis of 252,147 livebirths documented slightly decreased risk of SGA which varied little with mother’s and father’s occupation including agriculture. (San Jose 1991).

- The comparison of almost 200,000 births to farmers with those to nonfarmers (1967-1989) in Norway, revealed fewer SGA births among farmers when adjusted for the year of birth, maternal age and geographical region (Kristensen 1997).
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Discussion (6) – results of studies of women exposed to pesticides

• Three other studies related the risk of SGA to pesticide exposure.

• A case-control study in 29 hospitals in Shanghai, China, covered 75,756 infants with birthweights of ≥ 1000g. Women exposed to pesticides during pregnancy had an approximately threefold higher risk of having an SGA infant after adjusting for the sex of infant, fetal number, birth defects, maternal chronic illness and pre-eclampsia (Zhang 1992).
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• Based on the National Natality Survey and National Fetal Mortality Survey in US an elevated risk of delivering an infant with very low birth weight was found in the group of women reporting exposure to pesticides at work OR=2.4 95% CI(1.1.-5.0) (Savitz 1989).

• In a large sample of farming couples identified in the Canadian Census of Agriculture, preterm delivery was strongly related to the use of some herbicides (atrazine, 2,4-dichlorophenoxybutyric acid) (Savitz 1997). The exposure to pesticides was assessed for the male partners, however the authors did not exclude a possibility that the reported associations could have been due to maternal exposure as well.
As the exposure variables were obtained through an interview, one may consider the possibility of a recall bias. Mothers who had infants with a birth weight lower than that expected for a given duration of pregnancy may have recalled their activities in pregnancy in a more detailed manner than mothers from the control group.

*Do you think it could be a problem?*

*Do you think women would report the use of pesticides if in fact it as not true?*

- It is very unlikely that they would report the use of pesticides if they did not use these preparations on their farm.
- We analyzed the risk of other perinatal outcomes like prematurity and threatened abortion in relation to pesticide exposure. There was no indication that these events were reported more frequently by the subjects exposed to pesticides.
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Discussion (8) - biological plausibility

The negative effect of pesticide exposure on birthweight was found to be associated mainly to exposure to synthetic pyrethroids and to much less extend to phenoxyacetic acid derivatives. To date, pyrethroids have not been reported to exert any negative effect on the fetus.

How would you judge the biological plausibility of the results?

- The mammalian toxicity of these chemicals is regarded as low. However, chronic animal studies on pyrethroid toxicity indicate that they cause liver enlargement, accompanied by bile duct proliferation and focal necrosis of liver cells (Rose 1999).

- If hepatic impairment occurs in humans, this might be a mechanism by which pyrethroids could interfere with fetus alimentation.
Do you think that some other, not controlled in the analysis, confounding could explain the results?

- The study population was rather homogeneous in terms of socioeconomic status, age and nutrition habits.
- It was limited to uncomplicated pregnancies, and mothers with such diseases as diabetes, hypertension, infectious diseases and other pathology increasing the risk of intrauterine growth retardation were excluded from the study.
- All the examined women received prenatal medical care at local maternity units and it is unlikely that major maternal or fetal pathology went on unrecognized.


