NEONATAL OUTCOME FOLLOWING EXPOSURE TO ORGANOPHOSPHOROUS PESTICIDES

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Abstract — The aim of our study was to determine the neonatal outcome in mothers and children exposed to organophosphorous pesticides (OP). We found that 22.4% pregnant women were exposed to organophosphorous pesticides. OP pesticide concentration was higher in breast milk, newborn sera than maternal sera. Newborn parameters such as birth weight, birth length, head circumference, Apgar score and presence of meconium, as well as gestational age of delivery, showed no significant difference between the two groups. However, postpartum weight loss, hospitalization duration, levels of newborn bilirubin and glycaemia differed significantly between the two groups. Morbidity and presence of CNS disorders were six times and more than twelve times higher, respectively, in the OP-exposed than in the OP pesticide non-exposed group.

Key words: Organophosphorous pesticides, newborn morbidity, CNS disorders

INTRODUCTION

Maternal health and fetal growth in pregnancy are related to many environmental factors. Fetal growth depends on many factors such as a mother’s nourishment and her general health etc. The exposure of a pregnant woman to OP pesticides is harmful both to her and to her fetus. There is not much information about the possible effects of low-dose exposure to OP pesticides in our region. Potential adverse effects in the newborn may occur and these effects could be dose dependant. These effects and influences on the newborn depend on the duration, pesticide concentration and exposure of a mother to the toxicants.

The transfer of pesticides through the placenta, from mother to fetus, depends on different factors such as the mother’s sera concentrations, exchange of pesticides between the maternal and fetal blood, the surface area of the placenta, the thickness of the membrane barrier, etc (Ostrea et al., 2004, 2008).

The metabolisms of children and fetuses differ from that of adults since their organs continually grow and differentiate. These processes may be adversely affected by exposure to chemicals. Fetuses and children may be more or less susceptible than adults, depending on the pesticide to which they are exposed. There are many differences in the abilities of fetuses and children to absorb, distribute, deactivate, detoxify and excrete pesticides compounds (their metabolic rates are greater than those of adults). All of these can affect the toxicity of pesticides in referred age groups (IEH A Review, 2002).

Fetus organ malformations during in utero growth have lifelong effects on the growth and development of children (Fowden et al., 2004). Prenatal exposure to OP pesticides affects in utero and postpartum growth in many animals, e.g. reduced
birth weight and reduced postpartum weight gain. The stress effects of other pesticides (PCBs etc.) on birth weight and postpartum weight gain were investigated in school children (IEH A Review, 2002).

Some OP pesticides are neurotoxic. They cause aberrations in neuronal proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis. These problems affect the fetus and have been described in animals and humans exposed to these compounds (Lotti et al., 2005).

Chromosomal abnormalities, DNA damage, and predisposition for leukaemia were reported in infants born to mothers who were antenatally exposed to pesticides (Sánchez-Peña et al., 2004).

In humans, abnormal reflexes in newborn infants, as assessed by the Brazelton Neonatal Behavioural Assessment Scale, were associated with the exposure of a mother to environmental organophosphates during pregnancy (Engel et al., 2007).

The aim of our work is to investigate the influence in the newborn of low-level exposure to OP pesticides.

MATERIALS AND METHODS

Study design
In this randomized study we examined the presence and clinical manifestations caused by OP pesticides in children born to OP pesticide-exposed mothers. The control group was OP pesticide non-exposed mothers and newborn.

The study investigated only term singleton vaginal deliveries, breast milk feeding newborns, without any milk and/or liquid substitution.

Patients without any prenatal pregnancy control, heavy smokers (more than 10 cigarettes per day), mothers with serious chronic diseases such as diabetes, hypertension, thyroid disease or those who developed a serious pregnancy complication that could affect fetal growth and development were excluded. Furthermore, mothers who consumed more than one alcoholic drink (wine, beer, spirits) per day or who used illegal drugs were excluded. Children born with congenital malformations were also excluded.

This study was approved by the Institutional Review Board of the Clinical Centre Kragujevac. A total number of 600 prenatal patients were recruited in delivery rooms at Ob/Gyn Clinic, Clinical Center Kragujevac during 2006 and 2007. Of these, 87 mothers and newborn were excluded because of additional criteria such as medical complications, inability to collect biologic specimens and refusal to continue or to participate. The final group for this analysis consisted of 513 mothers and newborn. The experimental group consisted of 115 mothers and newborn exposed to OP pesticides, registered by sera levels, and the control group had 398 mothers and newborn not exposed to OP pesticides.

A preparation questionnaire was given to the mothers during their third trimester and/or during the parturition period to obtain information of potential pesticide exposures, maternal health, socio-cultural characteristics and lifestyle habits. We adapted potential covariances of measured variables, to avoid influence on the growth of newborn.

Parameters of fetal and neonatal growth
Information regarding fetuses was registered from the mothers’ and infants’ medical records, including date of delivery, gestational age at birth, sex, birth weight, length, head circumference, Apgar scores and presence of meconium. Newborn morbidity and therapy during hospitalization were analyzed too. We examined neonatal parameters related to metabolic functions such as glycaemia and sera bilirubin, postpartum weight loss, newborn morbidity, especially CNS disorders.

Blood and milk samples
Samples of maternal blood as well as newborn blood were collected 3 days after delivery by the
method of heparinized Vacutainer tubes. The tubes were centrifuged at 2400 rpm for 10 min and the plasma was removed. The plasma samples were kept at -70°C until analysis. Breast milk was also collected 3 days after birth, to avoid colostrum. Breast milk was also solid phase-extracted.

Samples were taken to the Medical Military Academy, Laboratory for Toxicology, National Toxicology Institution for further analyses.

Methods of laboratory tests including sampling, QC, reproducibility, limits of detection (LOD) were followed by many articles (Barr and Needham, 2002). In order to avoid any incidental exposures and to increase sensitivity and/or selectivity, we performed analyses according to the forensic application for standard diagnosis of acute pesticide intoxication in the National Toxicology Institution. Laboratory standards (native, internal, calibration), and sample preparation, quantification and method validation were managed through the standard procedure. Instrumental analyses were performed by GC Varian 3400 with integrated Varian 8100 auto sampler on 260°C, Varian Saturn II Ion Trap Mass spectrometer and PC Varian Saturn.

We analyzed OP pesticides such as malation, dimetoat, diazinon according to group identification. In order to calculate the combined effects of these OP pesticides, we used U.S. EPA for conducting cumulative risk assessment for organophosphates (U.S. Environmental Protection Agency, 2002). In short, all organophosphates were put into the most frequent diazinon equivalents based on the ratio of the relative potency factors calculated by the EPA. Malation and dimetoat levels in diazinon equivalents were evaluated by the diazinon levels using EPA methodology. Serum bilirubin and glycaemia were determined by a standard laboratory test and instruments.

CNS disorders were noted by clinical and ultrasound examinations. Clinical signs such as limb and axis hypertonia and hypotonia, tremor and hyperexcitability, consciousness disorders, hyperalerty, convulsions, nistagmus and ultrasound data for hemorrhage, ischemia encephalopathy, brain cysts and agenesis, dilatation of the brain ventricular system were diagnostic criteria for CNS disorders.

Statistical analysis

By appliance of Student’s t test and chi-square test, the results of certain parameters between the control and experimental groups were processed.

RESULTS

Our first finding is displayed in Figure 1 that shows the incidence of OP pesticide exposure in exposed and non-exposed groups. Overall OP pesticide concentrations in the sera were 22.4 % (115 of 513 mothers). Thus, the experimental group and control group (115 pairs of OP exposed and 398 OP non-exposed mothers and children, respectively) will be further tested in many correlations (Fig. 1).

The biochemical results presented in Table 1 show the relation between OP pesticide concentration in maternal blood sera and breast milk. The concentration of OP was higher in breast milk due to elevated pesticide concentrations in fat tissue. This was expected and correlated to indicate the transfer of the pesticide influences from OP-exposed mothers to their children (Table 1).

Table 1 shows the correlations of OP concentrations between maternal and newborn sera. According to the higher concentrations of pesticides in breast milk, it was expected that newborn

Figure 1. The incidence of OP pesticide exposure in exposed and non-exposed groups
sera concentrations of pesticides were significantly higher compared to OP concentrations in maternal sera (p = 0, 00078) (Table 2).

Overall correlations between the two newborn groups is shown in Table 3 as adjusted newborn parameters. As can be seen, there is no significant difference in Apgar score and presence of meconium. Newborn data such as birth weight, birth length, and head circumference showed no significant difference between the two groups (Table 3).

One of the adjusted parameters, gestational age of delivery, also showed no difference between the two groups. However, data such as postpartum weight loss, hospitalization duration, levels of newborn bilirubin and glycaemia were significantly different.

The presence or absence of morbidity in newborns is given in Table 4. Morbidity presence was highly correlated to the OP-exposed group. Infection symptoms and consequential treatments were followed by laboratory analyses (white blood count, CRP levels, body temperature). Treatment of high-level bilirubin was standard according to the levels of sera bilirubin (Table 4).

Table 1. Correlation between OP pesticide concentration (μg/dm³) in maternal sera and breast milk

<table>
<thead>
<tr>
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<th>N</th>
<th>min.</th>
<th>max.</th>
<th>mean</th>
<th>sd</th>
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</thead>
<tbody>
<tr>
<td>Maternal sera</td>
<td>115</td>
<td>0,50</td>
<td>2,67</td>
<td>1,29</td>
<td>0,115</td>
</tr>
<tr>
<td>Breast milk</td>
<td>115</td>
<td>0,65</td>
<td>3,23</td>
<td>1,64</td>
<td>0,155 *</td>
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</tbody>
</table>

* p = 0,00081

Table 2. OP pesticide concentration in maternal and newborn sera (μg/dm3)

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<th>N</th>
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<td>115</td>
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<td>1,29</td>
<td>0,115</td>
</tr>
<tr>
<td>Breast milk</td>
<td>115</td>
<td>0,45</td>
<td>3,15</td>
<td>1,62</td>
<td>0,186 *</td>
</tr>
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</table>

* p = 0,00078

Table 3. Adjusted data mean value ± SD between OP exposed and OP non-exposed newborn

<table>
<thead>
<tr>
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<th>OP exposed</th>
<th>OP non exposed</th>
<th>significance</th>
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<tr>
<td>Apgar score</td>
<td>8,87 ± 0,32</td>
<td>8,91 ± 0,23</td>
<td>NS</td>
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<tr>
<td>Meconium</td>
<td>12,1 %</td>
<td>14,2 %</td>
<td>NS</td>
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<tr>
<td>Adjusted birth weight (g)</td>
<td>3350 ± 397,8</td>
<td>3443 ± 390,9</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted birth length (cm)</td>
<td>53,8 ± 3,0</td>
<td>53,9 ± 2,8,</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted head circumference (cm)</td>
<td>33,8 ± 1,4</td>
<td>34,2 ± 1,3</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted gestational age (GW)</td>
<td>39,2 ± 1,7</td>
<td>39,3 ± 1,6</td>
<td>NS</td>
</tr>
<tr>
<td>Adjusted weight loss (g)</td>
<td>138,1 ± 19,6</td>
<td>172,2 ± 15,7</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Adjusted hospitalization duration (days)</td>
<td>8,1 ± 0,8</td>
<td>4,4 ± 0,6</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Adjusted newborn bilirubin</td>
<td>213,4 ± 9,7</td>
<td>191,2 ± 8,4</td>
<td>p&lt;0,01</td>
</tr>
<tr>
<td>Adjusted newborn glycaemia</td>
<td>2,1 ± 0,19</td>
<td>2,7 ± 0,16</td>
<td>p&lt;0,01</td>
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</table>

Adjusted for newborn sex and gestational age NS-non significant

Most CNS disorders were present alone or associated with other morbidity factors. Due to the lack of properly educated pediatricians for BNBAS scale of CNS disorders, we noticed and used general neurological signs such as rigidity, excitability etc. to determine newborn CNS morbidity. This special point showed one of the greatest differences between the OP exposed and OP non-exposed newborn groups.
The probability of aggregate morbidity presence in the OP pesticide exposed group was about six times higher than in the non-exposed group (OR = 5.71 95% CI 1,158 – 9,504) (Table 5).

In Table 6 we analyzed the probability of CNS disorders in the OP pesticide-exposed group vs. the non-exposed group of newborns. Our findings showed that the risks of developing some CNS disorders were about twelve times higher in OP pesticide-exposed newborn (Table 6).

DISCUSSION

We found that 22.4% of patients were exposed to OP pesticides. This was higher than other findings, implicating misuse of OP pesticides in Serbia (Ostrea et al., 2004).

As expected, OP pesticide concentrations registered in maternal sera were lower than in breast milk, in accordance with breast tissue active transport.

In addition to transplacental, another OP pesticide transfer was through breast milk. Therefore, sera concentrations were significantly higher in newborn than in mothers (Perera et al., 2003; Corrion et al., 2005). Incompetence of pesticide excretion in newborn metabolism is probably the main reason for higher concentrations (Whyatt et al., 2005; Barr et al., 2007).

Comparing anthropometric parameters between exposed and non-exposed newborn, we concluded that there are no differences in birth weight, birth length and head circumference. However, Diazinon exposed animals showed growth restriction; none of that showed on human newborn. In our study, we examined only term pregnancies, so there are no data about pesticide-induced IUGR (Whyatt et al., 2005; Zhao et al., 2005; Perera et al., 2005; Whyatt et al., 2004; Berkowitz et al., 2004).

In the group of parameters for neonatal outcome (Apgar score and presence/absence of meconium), there are no differences between the two groups, as concluded by other authors (Eskenazi et al., 2004).
Following the mother’s protective role during pregnancy, we registered increased newborn weight loss and prolonged hospitalization. We could not compare these data and we can recognize it by the partial immaturity of metabolism and GI disorders, such as hypoglycaemia and elevated bilirubin in OP-exposed newborn.

So far, acute high-level intoxication by OP pesticides is well known, but prolonged low-level exposure, such as in lactation are not so well recognized (Perera et al., 2003; Zhao et al., 2005). Newborn exposed to pesticides display oncogene and neurotoxic disorders as well as damage to the immunologic system (Berkowitz et al., 2004).

Investigating newborn morbidity between the two groups – exposed and non-exposed to OP pesticides – we observed a significantly higher incidence of different types of newborn morbidity, 61.1 vs. 32.1, respectively (Weselaka et al., 2007). Different indicators of morbidity were present, mostly as signs and symptoms of inflammatory processes, appearance and treatment of hyperbilirubinemia and hypoglycaemia. The most frequent findings were combinations of CNS disorders with other morbidity items.

Our results show that newborn exposed to OP pesticides had CNS consequences. Rice and Jerry (1982) claimed that brain development is programmed and other authors pointed out that pesticides damage the early phase of its development (Rice and Jerry, 1982; Rogan et al., Rosso et al., 2000).

Neural processes are managed by many neurotransmitters. Early breakage of neurons could be undiagnosed in a prolonged postnatal period but they can be registered as different neurological and psychological signs and symptoms of diseases (Eskenazi et al., 2007).

Some authors assert that OP pesticides are not only AcCh esterase inhibitors but they cause something more complex. Some brain regions are more sensitive to pesticide influence to G protein of adenyl cyclase and DNA transcription (Perera et al., 1999). Zhao et al., (2005) registered that neuron replication and differentiation was suppressed by pesticides, but the level of sera proteins, as protectors, is lower in fetuses and newborn, causing the severe damages of CNS. It is quite difficult to quantify every individual neurological defect, (Whyatt et al., 2005). Therefore, researches into CNS disorders were performed by neurological examination, brain Doppler ultrasound as well as laboratory parameters.

Due to the lack of properly BNBAS educated staff, (Canals et al., 2003; Ohgi et al., 2003; Gale et al., 2004), evaluation was performed by general neurological survey. Nevertheless, the presence of CNS disorders was 38.9%, vs 7.1% in the control, combined with other newborn morbidity (Young et al., 2005; Ricceri et al., 2006). OP pesticide liability increases the chance of newborn morbidity by almost six times (OR 5.71 95 %CI 1,158 – 9,504) and it increases CNS morbidity over twelve times (OR 12.8 95%CI 2,347 – 29,164).

**CONCLUSION**

We emphasize the higher level of morbidity and CNS disorders in newborn exposed to OP pesticide. Consequently, the improvement of pesticides exposure protection is a necessity. Besides pesticide detection in food, water, soil, air, pregnancy screening is important in imposing a better neonatal outcome (Ostrea et al., 2008; Whyatt and Barr, 2001; Megan et al., 2006; Ostrea et al., 2006). By examining maternal urine and milk a fast, non-invasive, inexpensive and reliable prediction of many postnatal infant problems is possible.

**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>OP</td>
<td>Organophosphorous pesticides</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<td>CI</td>
<td>Confidence index</td>
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<td>BNBAS</td>
<td>Brazelton Neonatal Behavioral Assessment Scale</td>
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<td>GW</td>
<td>Gestational Week</td>
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REFERENCES


Циљ ове студије је да одреди утицај органофосфорних пестицида (ОП) на труднице и новорођенчад након регистраованог ниског дејства. Регистровали смо појаву у 22,4% случајева експозиције трудница и новорођенчади. Концентрација органофосфорних пестицида је била виша у мајчином млеку, серуму новорођенчади него мајчином серуму. Параметри новорођенчади као што су тежина, дужина, обим главе, Апгар скор и присуство меконијума у плодовој води у току порођаја нису показовали разлике између две група. С друге стране, постпорођајни губитак масе, дужина хоспитализације, серумски нивои гликемије, билирубина су се значајно разликовали међу групама. Морбидитет и болести ЦНС су биле шест и више од дванаест пута више у органофосфорно изложеној него у неизложеној групи трудница и новорођенчади.