# Prolonged Exposure of Low Doses of Fipronil Causes Oxidative Stress in Pregnant Rats and Their Offspring

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**Citation**

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**Abstract**

Fipronil (FPN) is a phenylpyrazole class insecticide, which is widely used to control pests in agriculture, health and home. We have studied the effect of prolonged exposure to low doses of FPN on the state of lipid peroxidation and antioxidant protection of pregnant rats and their offspring. It was found that prolonged exposure to low doses of fipronil leads to oxidative stress in pregnant females and their offspring. In pregnant rats the maximum increase in the level of malone dialdehyde as the main indicator of lipid peroxidation, detected on 14-21 days of pregnancy. This was accompanied by a marked reduction in the activity of catalase, one of the important antioxidant defense systems. Similarly, fipronil caused by oxidative stress in the offspring, which had maximum expressed on 7-14 days after birth, in the milk feeding period. Although the degree of oxidative stress in the offspring decreases after cessation of receipt of the pesticide or its metabolites through of breast milk, the effects of fipronil-induced oxidative stress on the developing organism needs further extensive studies.

## INTRODUCTION

Fipronil (FPN) is N-phenylpyrazole insecticide that is widely used to control pests in agriculture, health, and household. FPN is registered for use to control pests of corn, cotton and rice in several parts of the world. The use of FPN-containing insecticides grew and continues to growdue to the limitation of the known organochlorine and organophosphorus pesticides (Tingle et al., 2003). Distinct advantages of FPN are that it is one of the most selective of the insecticidal blockers of the gamma-aminobutyric acid (GABA)-gated chloride channel with a favorable safety factor between insects and mammals and generally can be used at low and very low doses to achieve effective pest control (Hainzl and Casida, 1996). Recently FPN has also been found to block glutamate-activated chloride channels in cockroach neurons (Zhao et al., 2004). Since

mammals are devoid of this type of chloride channel, FPN block of the glutamate-activated chloride channel is deemed responsible, at least partially, for the higher selective toxicity to insects over mammals (Narahashi et al., 2010). Initially it was assumed that due to selective action FPN is a relatively safe for non-target organisms, including mammals and humans (Hainzl et al., 1998). However, subsequent studies have shown that phenylpyrazole insecticides, including FPN, may have high common cytotoxic effect for the vertebrates and mammals. The degree of toxicity depends on the dose, method and duration of exposure.Currently, there are sufficient data on the cytotoxic effect of FPN on the non-target organisms in vivo and in vitro.On in vitro models of SH-SY5Y human neuroblastoma cells was shown that FPN induces neuronal apoptosis, mediated by increased generation of reactive oxygen species, that is, through oxidative stress (Lee et al., 2011; Ki et al., 2012).In studies on in vitro models of rat neuronotypic pheochromocytoma PC12 cells had also been shown that FPN inhibited DNA and protein synthesis in undifferentiated cells and evoked oxidative stress to a greater extent than did chlorpyrifos, resulting in reduced cell numbers even though cell viability was maintained (Lassiter et al., 2009).Comprehensive treatment with antioxidants (vitamin E and ascorbic acid) helped to reduce the degree of oxidative stress, but did not prevent the loss of cells (Slotkin andSeidler, 2010).The toxic effect of FPN in vivo, mainly caused by oxidative stress, have also been detected in the rats, mice, carp fishes, green frogs and other animals (Ohi et al., 2004; Ferreiraet al., 2012; Clasen et al., 2012; Reynaud et al., 2012). A few cases of acute human poisoning by FPN have been also described

(Mohamed et al., 2004; Lee et al., 2010). In these cases,neurological symptoms (50%) were the most common, followed by ocular (44%), gastrointestinal (28%), respiratory (27%), and dermal (21%) symptoms (Lee et al., 2010). Thus, at the present time can be considered proved role of FPN in the development of oxidative stress and related toxic effects on the non-target organisms of the vertebrates and mammals. However, most of these studies were conducted on cell models in vitro, which have been applied sufficiently high concentrations FPN. The use of cell models in toxicology has its obvious advantages. However, it is not able to reveal many aspects of the problems associated with the exposure, persistence, metabolism and pharmacokinetics of the toxin (Lassiter et al., 2009).It should also be noted that in the wild animals and people often prone to prolonged exposure to low doses of pesticides. Studies over the past decades have challenged the traditional concepts in toxicology such dogma "the dose makes the poison", because some of the chemical substances can have effects at low doses that are not predicted by effects at higher doses (Vandenberg et al., 2012). Of particular concern are the toxic effects on pregnant women and infants because oxidative stress of developing organism can have harmful effects on the nervous system and lead to adverse neurological outcomes (Slotkin and Seidler, 2010).The authors emphasize that if the global objective is to replace more dangerous pesticides like the organophosphates with supposedly safer alternatives like FPN, there is the need to perform much more extensive examinations of the consequences of fetal and neonatal FPN exposure in vivo.The risk of contamination of the environment of fipronil is quite high.Recently studies J. Gan et al., (2012), have clearly established residential drainage as a direct source of pesticide pollution in urban waterways, and identified fipronil and its metabolites as a new and widespread pollutant with potential ecotoxicological significance.In this regard, of course, pregnant women and children represent a group at particular risk (Al-Gubory et al., 2010). Unfortunately, information on the status of lipid peroxidation and antioxidant defense system during long-term exposure to low doses of FPN, especially at the pregnancy of mammals, was publically unavailable. For this reason in the present work we are studied the effect of long term exposure of low doses of the FPN on the state of lipid peroxidation and antioxidant protection of pregnant rats and their offspring.

## MATERIAL AND METHODS

Chemicals

Fipronil (FPN) as a 4% emulsion concentrate (trade name "Vigor") received from the joint Uzbek-German Company "Euro-Team".

Laboratory animals

Experiments were performed on white adult virgin female rats Wistar weighing 150-170 grams and sexually mature male rats were used for fertilization. All rats were kept under controlled temperature (22 ± 3 C) and humidity (40-70%) with a 12 hour light-dark cycle. Animals were kept on a standard laboratory diet and given water without restriction.

Experimental protocol

Rats were acclimatized for one week prior to the start of experiment. Then the female rats were divided into two groups of 45 rats each. The first (treated) group of rats was administered *per os* through gavage diluted in saline FPN at the rate of 3,6 mg / kg / day. This corresponded to 1/100 of $LD_{50}$ of the drug. In this group of rats, the administration of the drug did not stop until the end of the experiments, i.e. prior to sacrifice.

The second (control) group in the same way received the same volume (0,4 ml/rat/day) of sterile saline. On 31 day of experiments female rats of both groups were combined with male rats for fertilization. Pregnancy was monitored by the presence of sperm in vaginal smears. After becoming pregnant females separated from males and placed in separate cages for future research. The parts of both group pregnant females were sacrificed at 14 and 21 days of gestation (GD 14 and GD 21) under light ether anesthesia. Other rats were sacrificed in the same way at 14 and 21 days after delivery (lactation day, LD 14 and LD 21). It should be noted that long-term administration of low doses of FPN did not lead to the appearance of overt symptoms of toxicity in experimental rats. Only a few rats were found mild lethargy and a slight decrease in motor activity. Offspring of females treated with FPN by the number and size did not significantly differ from controls. There are only a belated opening of the eyes and detachment of ears compared to control. Offspring from both groups of animals were sacrificed at 7, 14, 21 and 30 days (postnatal days, PND 7, PND 14, PND 21 and PND 30) after birth under light anesthesia with ether.

Preparation of liver homogenates for biochemical measurements

After sacrificing the liver was immediately removed, weighed and cleaned of extraneous tissue and rinsed with ice-cold saline solution. To obtain an extract of 1 g liver was homogenized in 10 ml of cold phosphate buffer solution (pH 7.4). The homogenate was centrifuged at 8000 rpm for 15 min at 4° C and the resulting supernatant was used for biochemical studies.

Determination of lipid peroxidation and enzymes activity in liver tissue

Biochemical determination of the status of lipid peroxidation and antioxidant enzyme levels was carried out by known methods adopted in our laboratories (Karimov et al., 2004). Briefly, the level of maleic dialdehyde (MDA) was determined on the basis of the reaction with thiobarbituric acid (TBA) to form a colored complex (TBARS), which is then calculated by spectrophotometry and expressed as nmol /mg protein. The activity of superoxide dismutase (SOD) were determined spectrophotometrically using nitro blue tetrazolium as an indicator reagent and expressed as unite /min mg protein.In determining the activity of catalase (CAT) as a substrate using hydrogen peroxide, activity of CAT expressed as $H_2O_2$ /min mg protein.

Statistical analysis

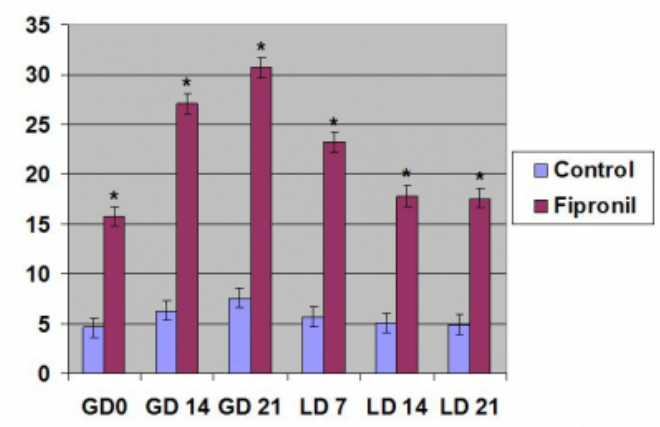
Calculation and statistical analysis was performed using the statistical package for Window`s. All data were represented as mean ± standard deviation (SD). Statistical significance between control and treated values were compared using Student's LSD test and P values less than 0.05 were considered significance.

## RESULTS

The obtained data are shown that prolonged exposure to low doses of FPN leads to significant induction of oxidative stress in female rats (Figure 1).In the female rats that before pregnancy during the month have received of FPN, the level of MDA was 3.4 times greater than in controls. A pregnancy itself has also contributed to a greater induction of oxidative stress. In the control rats that did not receive FPN, on 14 and 21 days of pregnancy there are only a slight increase in the MDA level compared to before pregnancy. In contrast, in the FPN treated females the levels of MDA in these days, respectively, were 4.3 and 4 times higher than the control values (P <0.001).After birth, the level of MDA, while generally somewhat lower, but still 3.5 - 4 times higher than the control values.

**Figure 1**

The level of lipid peroxidation ( MDA) in liver tissue of exposed to fipronil and control female rats in dynamic of pregnancy and lactation.




Note: * - the differences were statistically significant compared with control (P<0.05).

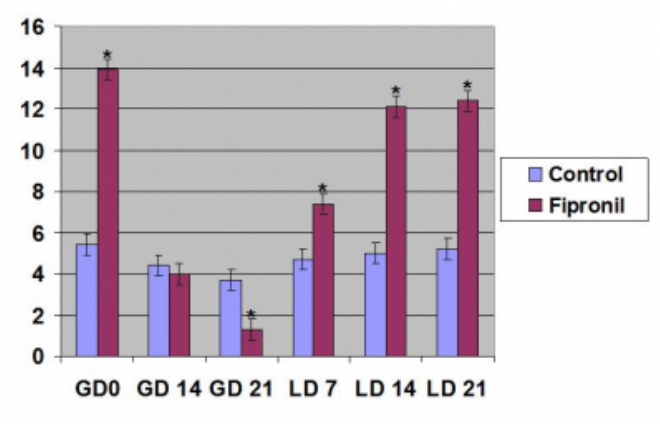
X-axis: GD - gestational day; LD -lactational day.

Y-axis: the level of maleic dialdehyde (MDA); nmol /mg protein.

Somewhat different results were obtained in studies of antioxidant enzymes (Figures 2 and 3).

**Figure 2**

The activity of antioxidant enzyme superoxide dismutase (SOD) in liver tissue of exposed to fipronil and control female rats in dynamic of pregnancy and lactation.



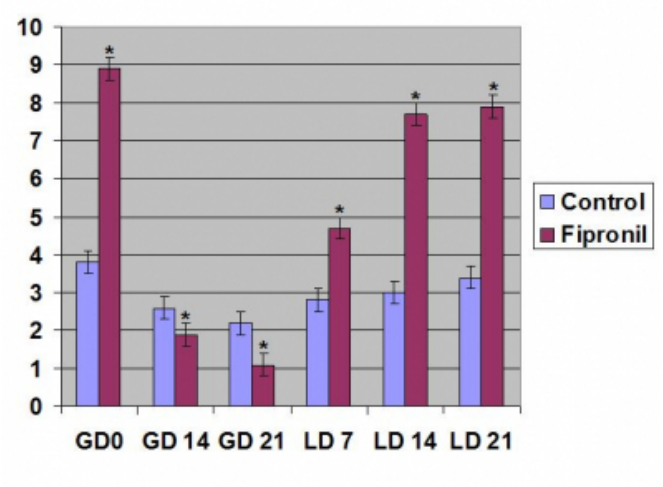

Note: * - the differences were statistically significant compared with control (P<0.05).

X-axis: GD - gestational day;LD -lactational day.

Y-axis: theactivity of superoxide dismutase (SOD); unite /min mg protein

**Figure 3**

The activity of antioxidant enzyme catalase (CAT) in liver tissue of exposed to fipronil and control female rats in dynamic of pregnancy and lactation.




Note: * - the differences were statistically significant compared with control (P<0.05).

X-axis: GD - gestational day; LD -lactational day.

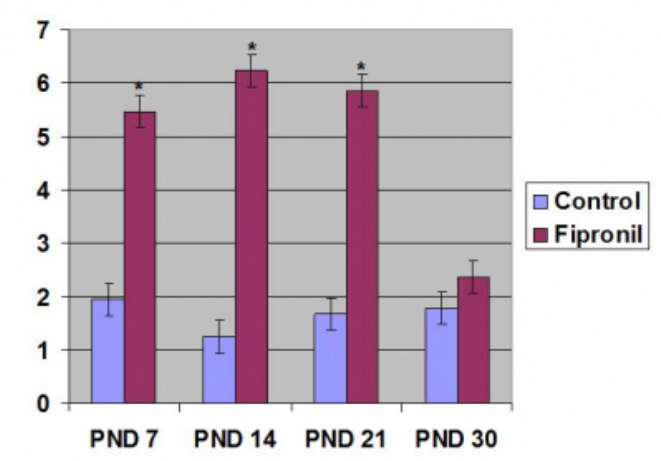
Y-axis: the activity of catalase (CAT); H2O2 /min mg protein.

The activities of SOD and CAT in the FPN treated female rats before pregnancy were more than 2.5 times higher compared with the control.At 14 days of pregnancy tended to reduce the activity of both enzymes, and on day 21 revealed their significant decrease compared with the control.After delivery the activities of these enzymes increased again and at 21 days of lactation were more than 2 times higher than the control values.

Prolonged exposure to low doses of FPN contributed significant induction of oxidative stress not only in the maternal organism, but also in their offspring (Figure 4).

**Figure 4**

The level of lipid peroxidation (MDA) in liver tissue of exposed to fipronil and control groups offspring in the dynamic of postnatal development.




Note: * - the differences were statistically significant compared with control (P<0.05).

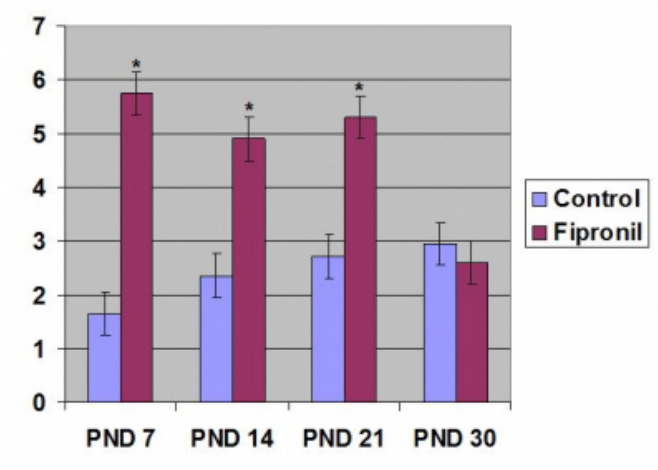
X-axis: PND – postnatal day.

Y-axis: the level of maleic dialdehyde (MDA); nmol /mg protein.

The level of MDA in the offspring of FPN treated rats progressively increased and the maximum value of MDA was observed on day 14 after birth. Subsequently the level of MDA gradually decreased and on day 30 after birth was not significantly different from controls.The activities of both SOD and CAT on 7 day after birth were more than 2 times higher comparative to control values (Figures 5 and 6).On day 14 revealed a decrease in the activities of both enzymes, although their activity remained

**Figure 5**

The activity of antioxidant enzyme superoxide dismutase (SOD) in liver tissue of exposed to fipronil and control groups offspring in the dynamic of postnatal development.



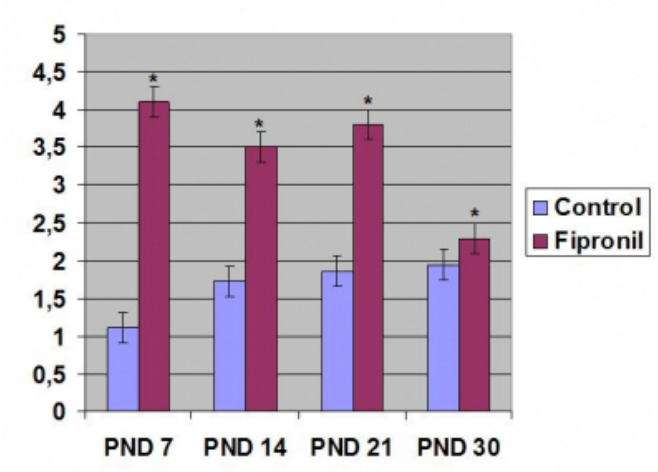

Note: * - the differences were statistically significant compared with control (P<0.05).

X-axis: PND – postnatal day.

Y-axis: the activity of superoxide dismutase (SOD); unite /min mg protein.

**FIgure 6**

The activity of antioxidant enzyme catalase (CAT) in liver tissue exposed to fipronil and control groups offspring in the dynamic of postnatal development.




Note: * - the differences were statistically significant compared with control (P<0.05).

X-axis: PND – postnatal day.

Y-axis: the activity of catalase (CAT); H2O2 /min mg protein.

significantly higher compared with the control.At day 21 again noted increased activity of both enzymes to approximately seven days after birth.On day 30 after birth the activities of both enzymes are decreased. However, in this time the activity of SOD was not different from control values, whereas the activity of CAT remained significantly elevated compared with controls.

## DISCUSSION

The induction of oxidative stress includes excessive production of reactive oxygen species (ROS and free radicals) as a result of an imbalance between the generation of ROS and antioxidant capacity. Antioxidant enzyme system protection features include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and others that may protect against the harmful effects of free oxygen radicals. Damage to membrane lipids, proteins and DNA is the final biomarker of oxidative stress caused by the action of many pesticides (Tuzmen et al., 2008). Induction of oxidative stress is one of the main mechanisms of action of many pesticides (Abdollahi et al., 2004; Amin and Hashem, 2012).Fipronil in certain doses and conditions is also a potent inducer of oxidative stress ((Slotkin and Seidler, 2010). In studies in vitro T.L. Lassiter et al., (2009), showed that the FPN is inherently more powerful violator of neurons than chlorpyrifos. Thus, cell differentiation increases susceptibility to fipronil-induced oxidative stress, although the antioxidant treatment provides no protection against the loss of cells. In models of human dopaminergic neuronal SH-SY5Y cells have been revealed that the FPN -induced apoptosis is mediated mainly through the generation of increased reactive oxygen species generation and activation of mitogen activated protein kinases (MAPK) members followed by activation of the intrinsic apoptotic pathway (Lee et al., 2011; Ki et al., 2012). The study in zebrafish embryos showed that the immature nervous system may be especially sensitive, with adverse effects on the structure and behavior of non-infringement of the gamma-aminobutyric acid (GABA) receptors (Stehr et al., 2006). B.Clasen et al., (2012) have investigated the antioxidant profile, oxidative stress parameters and growth in carp fish exposed to fipronil under rice field conditions. It was revealed that fipronil insecticides cause alterations in the biochemical parameters in different tissues of carp without affecting the growth or the survival of the fish.

Unfortunately, in the available literature we have not found

studies on the effect of FPN to pregnant mammals. According to our findings, the effect of FPN female rats for 30 days before pregnancy significantly increases oxidative stress. Pregnancy promotes further induction of oxidative stress.The most pronounced fipronil-induced oxidative stress detected on day 21 of pregnancy, when the MDA level many times higher than the control parameters.However, despite the high level of MDA, the enzymes activities both of SOD and CAT on 21 days of gestation revealed a significant decrease compared with the control group. Along with this, in the control group of rats in the development of pregnancy, we found a tendency to reduce the activity of antioxidant enzymes of liver compared to the before pregnancy.It is known that the physiological pregnancy is accompanied by the formation of large amounts of free radicals, mainly reactive oxygen species (ROS).High levels of ROS during embryonic, fetal and placental development are characteristic of pregnancy (Al-Gubori et al., 2010).Naturally, under these circumstances, the organism is forced to mobilize its antioxidant enzymes from the liver to other organs that need extra antioxidant protection, first of all, to the placenta.In our view, the decrease in activity of antioxidant enzymes in the liver of pregnant rats, most likely due to this redistribution.If at the normal pregnancy such reallocation can maintain a balance between ROS and antioxidant protection, the additional induction of fipronil leads to disruption of this balance and the development of oxidative stress, the most pronounced at the end of pregnancy.According to our data, the completion of delivery and removal of the placenta leads to a fairly rapid recovery of the activity of antioxidant enzymes in the liver itself. After birth, the activity of these enzymes again increased and on day 21 of lactation was significantly higher compared to control values.All this indicated that additional toxicity during pregnancy leads to an imbalance between pro-oxidant molecules, including reactive oxygen and nitrogen, and the antioxidant defense system, which can play a key role in the pathogenesis of various complications of pregnancy such as spontaneous abortion, recurrent pregnancy loss, and preeclampsia (Agarwal et al., 2005; 2012). In their view, exposures to environmental pollutants are of increasing concern, as they too have been found to trigger of oxidative stress and can lead to risk of pregnancy.However, when exposed to FPN, we found no reduction in the number of offspring, the threat of miscarriage or other obvious signs of pregnancy disorders.Offspring of females treated with FPN by the number and size did not significantly differ from controls.There are only a belated opening of the eyes and detachment of ears compared to control. But that does not mean that the fipronil-induced oxidative stress does not render on prenatal and postnatal development of the offspring. Epidemiological studies have showed that changes in neurobehavior, sexual development, the prevalence of asthma and allergies, and the growth curvesin many cases are associated with pollutant exposure at early life stages (Schoeters et al., 2011). Early postnatal period is most vulnerable to the action of environmental hazards (Ahmed et al., 2010; Ahmed, 2011).

In the offspring from mothers with long-term exposure to FPN, we have also observed a significant induction of oxidative stress.The level of MDA progressively increased in the offspring, and its maximum value was observed at day 14 after birth.Then the level of MDA gradually decreased and on day 30 of postnatal period was not significantly different from controls.This means that oxidative stress, which arose as early as the embryonic period, continues to develop in postnatal life.The degree of oxidative stress decreases as the cessation of receipt of a pesticide or its toxic metabolites in breast milk.This explains the decrease of MDA level and activity of SOD and CAT on 30 day after birth, when completely stopped the intake of FPN or its metabolites.

Oxidative stress during pregnancy may play a key role in the pathogenesis of many diseases of offspring, which could be observed in the different periods of postnatal life (Valko et al., 2007; Al-Gubory et al., 2010; Schoeters et al., 2011).Our study clearly showed the development of pronounced oxidative stress in the offspring when the mother's body exposed to low doses of FPN during pregnancy and lactation. Sure, prenatal and early postnatal oxidative stress can cause a variety of violations of the further development of organs and systems, especially the nervous, endocrine, reproductive and immune systems of offspring. Our results support the view of other researchers and point to the need to perform much more extensive examinations of the consequences of fetal and neonatal FPN exposure in vivo (Slotkin and Seidler, 2010). It is important to develop effective methods of prevention and treatment of possible future adverse effects of prenatal and postnatal exposure to fipronil on the offspring. Recently, there has been a good deal of interest in the potential for the use of antioxidant therapies in the perinatal period to protect the fetus, particularly the developing brain, against oxidative stress in complications of pregnancy and birth (Miller et al., 2012).

Several studies have shown that the use of ascorbic acid (Fetoui et al, 2009, 2010), alpha-tocopherol or vitamin E (Amin, Hashem, 2012) leads to a reduction in oxidative stress when exposed to pyrethroid pesticides and restores the activity of enzymatic antioxidant defense systems.Recent studies have revealed that kaffeic acid and quercetin are also able to protect against oxidative stress and genotoxic effects of pesticides in vitro (Abdallah et al., 2012).Although we have not studied the effect of antioxidants during pregnancy under the impact of FPN, these data indicate the feasibility of antioxidants in pregnant women with potential risk to exposure of pesticides and its metabolites.This is particularly applies to well known and readily available medicines such as ascorbic acid and alpha-tocopherol.All this suggests that the conduct of an effective antioxidant therapy to pregnant women during both of pregnancy and lactation periods may indirectly prevent or reduce the degree of oxidative stress in the offspring.

In conclusion, it should be noted that prolonged exposure to low doses of fipronil leads to oxidative stress in pregnant females and their offspring. The highest level of lipid peroxidation detected during pregnancy is associated with reduced activity of antioxidant enzymes (SOD and CAT). In the offspring a more high level of oxidative stress had observed in the period of lactation. Although the degree of oxidative stress in the offspring decreases after cessation of receipt of the pesticide or its metabolites through of breast milk, the effects of fipronil-induced oxidative stress on the developing organism needs further extensive studies.

## References

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