Effect Of Prolonged Exposure Of Low Doses Of Fipronil On Thyroid Function Of Pregnant Rats And Their Offspring

K R Tukhtaev, N B Zokirova, S K Tulemetov, N K Tukhtaev, M R Tillabaev, O K Amirullaev, A N Otajonova, O O Yarieva

Citation

K R Tukhtaev, N B Zokirova, S K Tulemetov, N K Tukhtaev, M R Tillabaev, O K Amirullaev, A N Otajonova, O O Yarieva. *Effect Of Prolonged Exposure Of Low Doses Of Fipronil On Thyroid Function Of Pregnant Rats And Their Offspring*. The Internet Journal of Toxicology. 2013 Volume 10 Number 1.

Abstract

Fipronil (FPN) is a phenylpyrazole class insecticide, which is widely used in households, agriculture, and health care in the many countries of world. The effect of prolonged exposure to low doses of FPN on the state of thyroid hormones (TH) thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH) of the pregnant rats and their offspring have been studied. In non-pregnant female rats FPN exposure for 30 days before pregnancy resulted in a moderate decrease of levels both of the TH, but it significantly increased the concentration of TSH. The impact of FPN in pregnant female rats, and especially in lactating period, led to a marked reduction in TH concentration with a significant increase in TSH levels. It was also found that prenatal and postnatal exposure to low doses of FPN significantly disturbed thyroid function in the offspring. The concentrations both of T4 and T3 in all periods of experiments in the offspring from FPN-exposure rats were decreased, while the TSH level was significantly higher than controls. The hypothyroidism in the offspring was kept on 30 day of postnatal life, when delivery of FPN or its metabolites through the breast milk has been discontinued. It is probably may be due to a prolonged retention of the fipronil sulfone, as a more stable metabolite of fipronil, in the organism of offspring. The mechanisms of disorders of thyroid function of exposed to FPN pregnant females and their offspring has not yet fully understood and need further detailed investigations. The results of these studies may help to prevent the adverse effects of environmental pollutants on the thyroid function of pregnant women and their children.

INTRODUCTION

For more than two decades in the world literature uses the term “endocrine- disrupting chemicals or endocrine disruptors” (EDCs, or EDs), adopted by the Agency for the U.S. Environmental Protection Agency in 1991 (Boas et al., 2006; Diamanti-Kandarakis et al., 2009; Zoeller, 2010).This term brings together all chemical substances which in the humans and animals changing processes of synthesis, secretion, transport, and metabolism of natural hormones and thereby leads to a violation of hormonal homeostasis (Hotchkiss et al., 2008).Endocrine-disrupting effects have many pesticides, including the latest generation, and household chemicals and some medicines.There are more than one hundred pesticides of which 80% are commonly used as insecticides and fungicides, have different endocrine disrupting effects (Mnif et al, 2011). One of the most common representatives of the pyrazole class pesticides is fipronil (FPN), which as insecticide has a high efficiency even at low doses and is widely used in households, agriculture, and health care in the many countries of world (Tingle et al., 2003). The risk of contamination of the environment to fipronil, and especially to its more toxic and stable metabolite a fipronil sulfone is big enough, because the use of FPN-containing insecticides continues to grow due to the limitation of the known organochlorine and organophosphorus pesticides (Lassiter et al., 2009). Recently studies M.P.Ensminger et al., (2013) showed an abundance of pesticides in surface waters of California. In water samples, all of the malathion,fipronil and -cyhalothrin detections, and most of the fipronilsulfone detections were above their lowest US EPA aquatic benchmark.It should be understood that even low doses of these pollutants may have endocrine - disrupting effects. Whether low doses of EDCs affect influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities (Vandenberg et al., 2012).

However the endocrine-disrupting effect of FPN was

discovered relatively recently and the reports on this problem are relatively rare.Early reports on the inhibitory effect of FPN on the thyroid gland appeared in the late 90's (Hurley, 1998).The author is suggested that the anti-thyroid effect of fipronil seem due to enhance the hepatic metabolism and elimination of thyroid hormones. M.Ohi et al. (2004) have studied the effect of a single exposure to high concentrations (70, 140 and 280 mg / kg) of fipronil on reproductive function and showed that fipronil may change the normal functioning of the endocrine system and cause adverse reproductive effects in female rats.Subsequent studies have shown that fipronil may act as the thyroid disruptor in the rats (Leghait et al, 2009).It was found that fipronil increases the clearance of thyroxine (T4) and this effect is associated with a high plasma concentration of fipronil sulfone, the fipronil main metabolite in several species including rats and humans.Unlike rats in sheep the effect of this treatment was limited to a moderate increase in free T4 clearance (Leghait et al, 2010).The authors are believed that the differences between rat and sheep for the potential of fipronil as a thyroid disruptor might be related to the difference in the exposure to the toxicant, the actual exposure to the sulfone metabolite of fipronil being lower in sheep than in rat. Recently studies have shown that the fipronil biotransformation into fipronil sulfone by hepatic cytochromes P450 (CYP) could act as a potential thyroid disruptor (Roques et al., 2012). It was showed that the fipronil sulfone treatment could reproduce the fipronil treatment effects on T4 clearance and hepatic enzyme induction in rats. The potential of fipronil sulfone to act as a thyroid disruptor is all the more critical because it persists much longer in the organism than fipronil itself (Roques et al., 2012).

Thyroid-disrupting effect of fipronil and its metabolites are probably not limited to the increase in the clearance of thyroxine (T4) and triiodothyronine (T3).M. Ferreira et al. (2012) showed that in the mice exposed to fipronil were detected significant changes in thyroid tissue structure with evident disorganization of the thyroid follicles, and changing the chemical composition of a colloid in the form of reduced protein.These data indicate that fipronil and its metabolites may have a direct toxic effect on the thyroid gland. Thus, at the present time there are conclusive proofs that fipronil, and especially its metabolite fipronil sulfone, have a pronounced thyroid-disrupting effect.

It should be noted that all of the studies of impacts of FPN on thyroid function were performed on adult specimens.However, animal and human organisms are most sensitive to the action of pesticides in embryonic and early postnatal life (Birnbaum and Fenton, 2003; Goldman et al., 2004).Moreover, the negative impact of this exposure may not manifest itself immediately, but after many years, often in the adult organism (Mnif et al., 2011).Maternal thyroid hormones play a crucial role in the development of organs and systems of the embryo and newborn (Ahmed et al., 2008; 2010).Hypothyroidism in pregnant women caused by thyroid-disrupting effects of xenobiotics can lead to unexpected disturbances during prenatal and postnatal development of the offspring (Ahmed, 2011). It is possible that the lack of thyroid hormones in the mother can lead to violation of the development of the fetal thyroid gland, thereby causing secondary hypothyroidism in newborns. Unfortunately, the thyroid-disrupting effects to exposure of FPN in utero and early childhood period are still virtually unknown. Based on the foregoing in the present work we have studied the effect of long-term exposure to low doses of fipronil on the state of thyroid function of pregnant female rats and their offspring.

MATERIAL AND METHODS

Chemicals

Fipronil (FPN, 5-amino-1-(2, 6-dichloro-4- (trifluoromethyl) phenyl)-4-(trifluoromethyl sulfinyl) pyrazole-3-carbonitrile) 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.

Determining of the level of hormones

After sacrificing blood was collected into dry sterile tubes without anticoagulant and the resulting serum was used to determine the concentration of hormones. The thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (thyrotropin, TSH) in serum were measured by immunoassay using specific kits of "Human" (Germany) and the spectrophotometer "Singl" (Germany). Thyroxine, triiodothyronine were expressed in ng / ml, and thyroid-stimulating hormone was expressed as mIU / l.

Statistical analysis

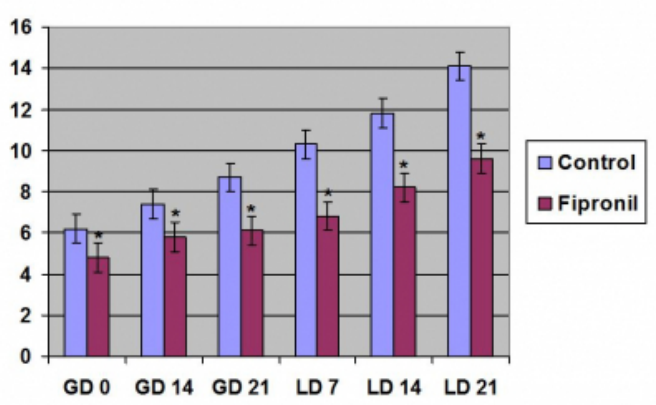
Calculation and statistical analysis was performed using the statistical package for Window`s. All data were represented as mean ± standard deviation (SD). Statistical significance between control and treated values were compared using Student's test and P values less than 0.05 were considered significance.

RESULTS

The obtained data showed that the pregnancy, especially lactation by itself lead to a progressive increase of T4 and T3 (Figures 1 and 2).

Figure 1

The concentration of thyroxine (T4) at pregnancy and lactation



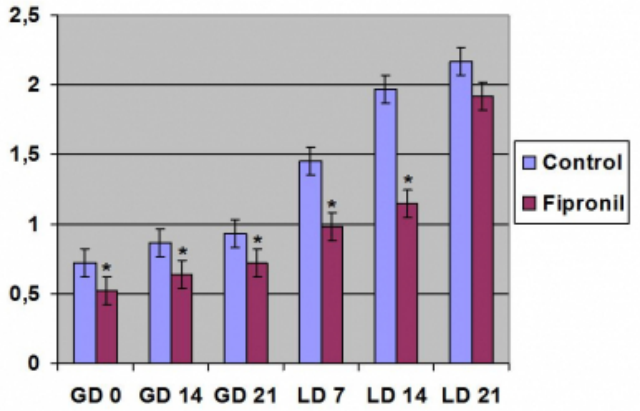
Note: * - the differences were statistically significant compared with control (P<0.05).

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

Y-axis: the level of thyroxine (T4); ng / ml.

Figure 2

The concentration of triiodothyronine (T3) at 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 triiodothyronine (T3); ng / ml.

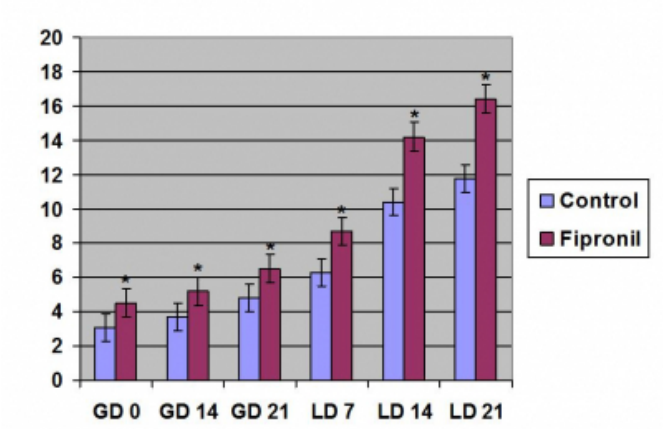
In the female rats that administered during the month before pregnancy of FPN, the concentration of these hormones although decreased slightly, but was significantly different from controls.Consequently, the 30 day exposure of low doses FPN is expressed the moderate hypothyroidism in non-pregnant rats.Nevertheless, even a small decrease in T4

and T3 in these animals was accompanied by an increase in the concentration of TSH (Figure 3). With the onset of pregnancy in rats receiving FPN has developed a distinct hypothyroidism. At 14 and 21 days of gestation the concentration both of T4 and T3 were significantly lower compared with the control.A particularly large difference between the FPN received and control rats has revealed at 14 and 21 days of the lactation. At these days the concentration of T4 and T3 in the FPN received rats on 1.4 - 1.47 times decreased compared with controls.The concentration of TSH of both groups of rats progressively increased from pregnancy to lactation.

However, the level of this hormone in FPN treated rats on all days of pregnancy and lactation significantly exceeded the corresponding parameters of the control group (Figure 3).

Figure 3

The concentration of thyroid-stimulating hormone (TSH) at pregnancy and lactation



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

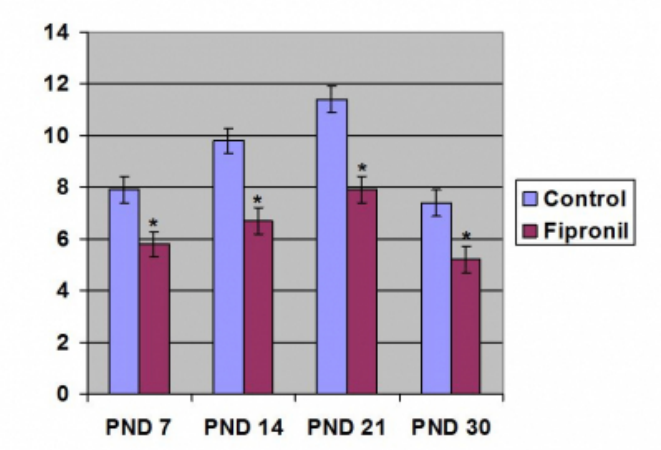
X-axis: GD - gestational day; LD -lactational day.

Y-axis: the level of thyroid-stimulating hormone (TSH); mIU / l.

The exposure of FPN in utero and through breast milk led to a significant breach of thyroid function in the offspring. The concentration both of T4 and T3 in FPN administered rats in all periods of the study were significantly decreased compared with controls (Figures 4 and 5).

Figure 4

The levels of thyroxine (T4) of the offspring at postnatal development



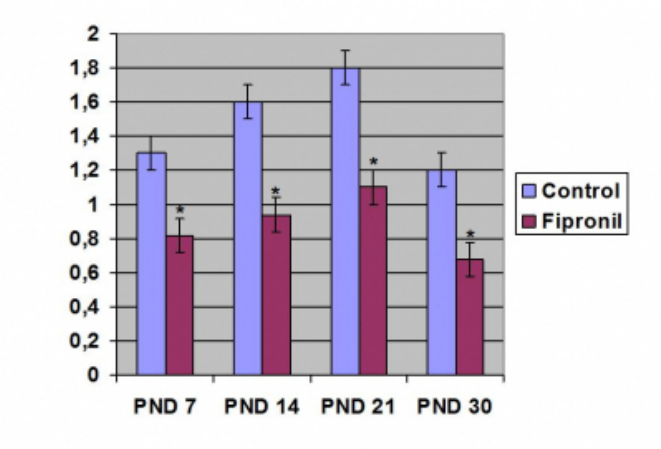
Note: * - the differences were statistically significant compared with control (P<0.05).

X-axis: PND – postnatal day.

Y-axis: the level of thyroxine (T4); ng / ml.

FIgure 5

The levels of triiodothyronine (T3) of the offspring at postnatal development.



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

X-axis: PND – postnatal day.

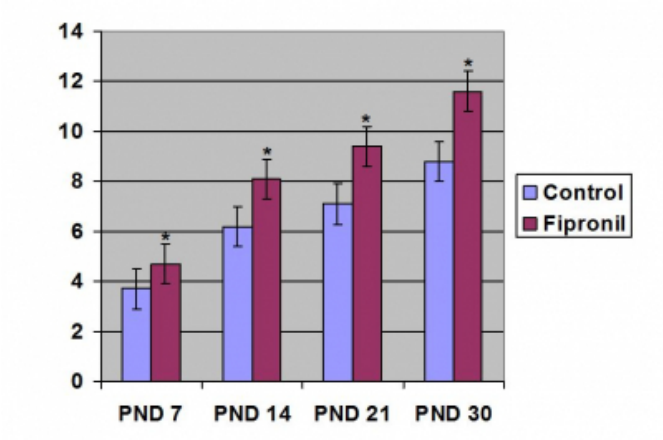
Y-axis: the level of triiodothyronine (T3); ng / ml.

The biggest difference in the concentrations of hormones has found on 14 and 21 days of lactation, when the level of hormones in the FPN administered rats was reduced by more than 1.4 times compared with control. On day 30 of

postnatal period the concentration of T4 and T3 in FPN received rats remained significantly lower compared with control. Determination of the concentration of the thyroid-stimulating hormone (TSH) showed its progressive increase on all days of the study. The maximum increase of TSH (to 1.3 time compared with control) was observed on 30 day after birth (Figure 6).

Figure 6

The levels of thyroid-stimulating hormone (TSH) of the offspring at postnatal development



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

X-axis: PND – postnatal day.

Y-axis: the level of thyroid-stimulating hormone (TSH); mIU / l.

DISCUSSION

Our study showed a gradual increase in the levels of thyroxine (T4), triiodothyronine (T3) and thyroid-stimulating hormone (TSH) in all female rats during the pregnancy and lactation. It is known that during pregnancy the need for thyroid hormones (TH) increases by 30-50 % and the thyroid gland has to cope with this increased demand (Gartner, 2009). This state may be a reflection of increasing the transfer of TH from pregnant females to their fetuses and / or more efficient production TH by the thyroid gland after birth (El - Bakry et al., 2010). The gradual increase in serum TSH is necessary for the development of thyroid gland in the sensitive embryonic period (Ahmed et al., 2008, 2010). Our data support the view that the normal functioning of the hypothalamic-pituitary-thyroid axis of the maternal organism is a necessary condition for the natural development of the thyroid gland of the embryo and for prevention of various disorders in the postnatal period. It is assumed that TSH may also play a significant role in the regulation of growth and development of the whole organism, providing communication to the growth hormone (GH) functions (Ahmed, 2011).

On our data prolonged exposure to low doses of FPN had different effects on thyroid function of pregnant and non-pregnant female rats. We found that in non-pregnant female rats FPN exposure for 30 days before pregnancy resulted in a moderate decrease of levels both of the TH, but it significantly increased the concentration of TSH. In contrast, the impact of FPN in pregnant female rats, and especially in lactating period, led to a marked reduction in TH concentration with a significant increase in TSH levels. Thus, our data suggest a thyroid- disrupting effect of prolonged exposure to FPN during pregnancy and lactation. Without a doubt, the lack of TH in the maternal organism leads to violations of the growth and formation of various organs, primarily the nervous and endocrine systems in the embryonic and early postnatal periods. Unfortunately, data about the effect of prolonged exposure to FPN through maternal organism on the postnatal growth and development of the offspring, we have not found. However, a number of studies have shown that hypothyroidism in the maternal organism, caused by the action of some environmental toxicants such as dioxins leads to the development of neuro-endocrine disorders in the offspring.Hypothyroidism in the mother's has a dose-dependent manner, and above all, accompanied by a decrease in TH and GH concentrations in the offspring, whereas TSH levels are significantly increased (Ahmed, 2011). Yu et al., (2009) have shown that prenatal and postnatal exposure of perfluorooctane sulfonate (PFOS) leads to development hypothyroidism in both of mothers and offspring. The authors suggest that in utero PFOS exposure and its postnatal accumulation, especially though maternal milk, are matters of great concern. However, not all pesticides with prenatal exposure lead to disruption of thyroid function in the offspring. For example, a widely used thiocarbamate insecticide mancozeb in the pregnant rats caused a pronounced hypothyroidism, while the weight, structure and function of the thyroid gland of offspring remained without significant changes (Axelstad et al., 2011).

We have found that prenatal and postnatal exposure to low doses of FPN significantly disturbed thyroid function in the offspring. The concentrations both of T4 and T3 in all periods of experiments in the offspring from FPN received

rats were decreased, while the TSH level was significantly higher than controls. It should be noted that the reduced level of thyroid hormones in a sufficiently high concentration of TSH was maintained on 30postnatal day when delivery of FPN or its metabolites through a breast milk had been stopped. This is probably due to a prolonged retention of the fipronil sulfone in the offspring, which persists much longer in the organism than fipronil itself and continues to act as a thyroid disruptor (Roques et al., 2012). Therefore, the FPN has a fairly pronounced thyroid-disrupting effect in the pregnant and its impact in the embryonic and early postnatal periods creates a risk for further growth and development of the child.

The mechanism of thyroid-disrupting effect of FPN, like all the other phenylpyrazole classes of pesticides, still has not fully understood. In a series works of French scientists have shown that a reduction in thyroid hormones (TH) is the result of increased clearance of TH by activated liver enzymes (Leghait et al., 2009; 2010). Furthermore, it was also proved that fipronil biotransformation into fipronil sulfone by hepatic cytochromes P450 (CYP) could act as a potential thyroid disruptor. The potential of fipronil sulfone to act as a thyroid disruptor is all the more critical because it persists much longer in the organism than fipronil itself (Roques et al., 2012). On the other hand, fipronil in certain doses can induce of oxidative stress with accumulation of reactive oxygen species (Abdollahi et al., 2004; Lassiter et al., 2009; Slotkin and Seidler, 2010; Ki et al., 2012). It is possible that in the mechanism of thyroid-disrupting effect of fipronil may play a key role these toxic products of oxidative stress, which may have a direct cytotoxic effect on the thyroid gland (Ferreira et al., 2012). Probably right C.Schmutzsler et al. (2007), who consider that the action of endocrine disruptors (EDs) is not fit into the classical scheme of hormone-dependent regulation and feedback. In their view, the effect of EDs is associated with complex multi-target and multi-modal actions on the hypothalamic-pituitary-thyroid axis. Although currently very little is known about the relationship with the concentration of thyroid hormones (TH) and growth hormone (GH), but it is can not exclude the participation of GH in the development of hypothyroidism in mothers and their offspring when exposed to endocrine disruptors (Ahmed, 2011). All this suggests that the mechanisms of disorders of thyroid function in exposed to FPN pregnant females and their offspring remain complex and need further detailed studies. The results of these studies may help to prevent the adverse effects of environmental pollutants on the thyroid function of pregnant women and their children.

In conclusion, it should be noted that prolonged exposure to low doses of fipronil leads thyroid-disrupting effect in pregnant rats and their offspring.The most pronounced hypothyroidism in mothers was revealed at the end of pregnancy and during lactation.The offspring hypothyroidism was detected in the milk feeding period and it was kept on 30 day of postnatal life, when delivery of FPN or its metabolites through the breast milk has been discontinued. This is probably due to a prolonged retention of the fipronil sulfone in the organism ofoffspring. The mechanisms of disorders of thyroid function in exposed to FPN pregnant females and their offspring has not yet fully understood and need further detailed investigations.

References

Author Information

Kadir R. Tukhtaev
Department of Histology, Embryology and Medical Biology, Tashkent Medical Academy
Uzbekistan

Nargiza B. Zokirova
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan

Sabirjan Kh. Tulemetov
Department of Anatomy, Tashkent Pediatric Medical Institute
Uzbekistan

Nodirbek K. Tukhtaev
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan

Mansur R. Tillabaev
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan

Olimjon K. Amirullaev
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan

Aziza N. Otajonova
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan

Oynisa O. Yarieva
Central Scientific-Research Laboratory, Tashkent Medical Academy
Uzbekistan