Toxic effect of fipronil on rats of different age

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Abstract

The toxic effect of fipronil on rats of different age groups (1-, 6-, 12-month age) at 10 mg/kg bw at acute and subacute exposure has been studied. It was demonstrated, that the hepatotoxic effect of fipronil caused the induction of cytochrome P450 and activation of LP processes. Fipronil influence on rats at acute and subacute causes in a liver of all age groups a circulatory disorders, a fatty dystrophy and stellate cells of liver (Kupffer's cell) activation. It was noted, that the degree of pathological changes increased with exposure time. Very sensitive animals to fipronil were rats in the age of 1 and 12 months. Hepatotoxic effect of fipronil is caused by induction of cytochrome P450 and activation of LP processes. It was shown, that the content of lipid peroxides and malonic dialdehyde in rat livers after subacute exposure was significantly increased than after acute fipronil exposure and in control. The level of cytochrome P450 in rat livers was significantly below after subacute fipronil exposure comparing with acute fipronil exposure, that determined LP activation and the progress of fatty degeneration and necrobiotic processes.

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Introduction

Due to intensification of environmental pollution processes by ecologically hazardous factors of physical, chemical and biological nature, which impact all components of ecosystem, thorough investigation of potential toxic effects on living organisms including human acquires special importance. Majority of these factors are products of human activities. According to Chemical Abstracts Service (CAS) 33 565 050 organic and inorganic compounds were registered on 15th of January 2008, however on 16th of October 2004 the number of compounds comprised 24 404 089, and on 14th of January 2002 – 19 14 111 [1]. These data suggest that increase in number of chemical compounds, which are introduced into the environment by man, the probability of its pollution by mutagenic factors also increases. Number of published data suggest that doses of various pesticides, used in agriculture affect like mutagens, causing cytotoxic and negative genetic effects [2, 3, 4]. According to toxicologist's study presence of pesticides was determined in many food types, and the number of pesticide poisoning according to WHO data comprises 1% annually [World Health Organization, WHO]. Pesticides could persist in soil for a long of time. They migrate and pollute surface and underground water sources. As a result of pesticide influence the the following occurs: disruption of population content of agro- and bio-cenosis, as well as elimination of natural predators and pests, negative effects on useful fauna, occurrence of pesticide resistant species populations, due to accumulation of trace concentrations of pesticides in agricultural crop types harvest quality may change, also it may cause negative influence on genetical material of all living organisms [5]. However pesticides application in various sectors of the human economical activity is inevitable, as it due to economic necessity.

Currently, in Kazakhstan against large number of grasshoppers new classes of insecticides, which comprise the class of phenylpyrazole and main active component of which is fipronil, are used [6]. In natural conditions and in mammalian organisms it can degrade and form toxic metabolites, which are characterized by high toxicity and stability in contrast to former chemical [7].

Data on toxic effects of fipronil on mammals are quite controversial and there is almost no data on its effects on organism during the postnatal ontogenesis. Therefore conduction of analysis of toxic effects of insecticides, which are based on fipronil, on mammalian organism during the ontogenesis is very important.

The purpose of the given work was to study cytotoxical effects of fipronil on experimental animals of different age groups.

Materials and methods

Studies of toxic effects of fipronil were performed on rats (*Rattus norvegicus*) (n=45) of different age groups (1-, 6-, 12-month age). Animals were divided into 9 groups (n=5 rats in each group): I-III – intact animals of three age groups; IV-VI - animals of three age groups exposed to aqueous solution of fipronil *per os* in single dosage of 10 mg/kg (acute exposure); VII-IX - animals of three age groups exposed to aqueous solution of fipronil *per os* for 10 days in dosage of 10 mg/kg (subacute exposure). The dosage was chosen according to available publications regarding oral LD50 of fipronil for rats (1/10 LD50) [WHO, Classificaation of Pesticides by Hazards 1998-1999, In ternational Programme on Chemical Safety, WHO/IPCS/98.21.]. Oral introduction of xenobiotic was carried out by special intragastric sonde.

Cytological specimen preparations were performed using standard protocol [8]. Histological sections were stained with hematoxylin-eosin. Slides were analyzed and photographed by Axioscope-40 (Zeiss) light microscope.

To determine the level of primary and secondary products of LP level in rat livers the spectrographic method was used [9, 10].

Cytochrome P450 content in rodents liver was determined according to Matsubara protocol [11]. Oxidized and reduced cytochrome P450 forms were determined by spectrographic method (Specord UV V15) relying on light absorption (wavelength spectrums - 450 and 490 nm). Protein concentration was measured using Bradford assay [12].

Statistical analysis for evaluation of all quantitative data standard Student's test were used. In order to obtain mean values of parameters were treated by standard methods of variational statistics [13].

Results and discussion

The observation of animal behavior demonstrated anxious behavior of rats independent from ages after acute and subacute fipronil exposure. Appetite loss, convulsions were demonstrated after subacute fipronil exposure.

The microscopic examination of intact rat livers of different age did not reveal any pathological changes (Fig. 1, 2, 3).

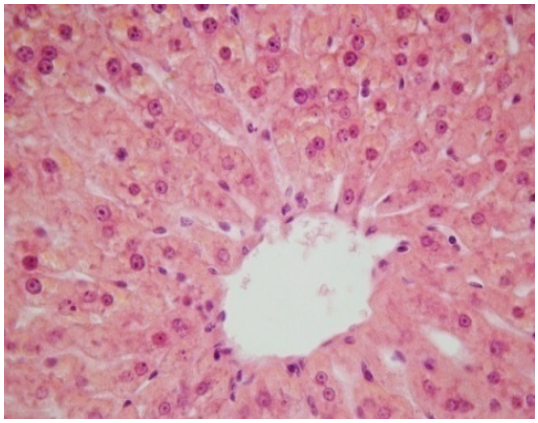


Fig. 1. Liver of intact 1 month age rats
(x 200).

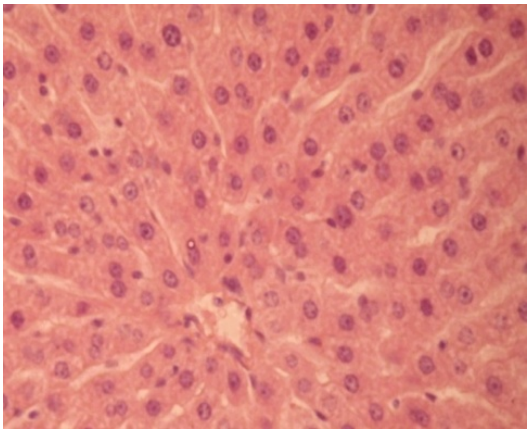


Fig. 2. Liver of intact 6 month age rats
(x 200).

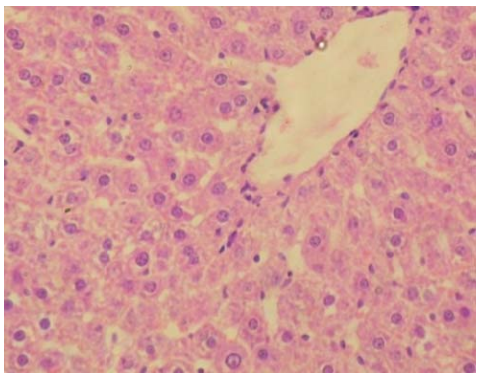
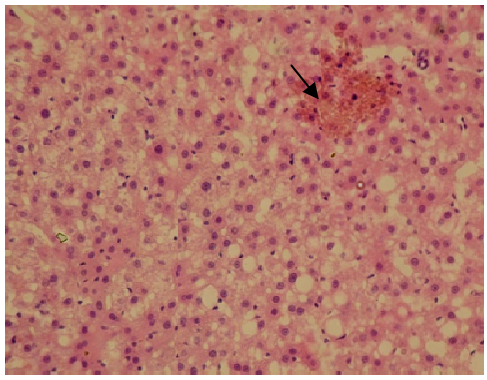


Fig. 3. Liver of intact 12 month age rats
(x 200).



After acute exposure of rats (one-mounth of age), fipronil caused the circulatory disorders in the form of perisinusoidal and Disse spaces dilations. Agglutinate erythrocytes were visible in sinusoids. Small fat drops were observed in the cytoplasm of particular hepatocytes (Fig. 4).

Diffusely distributed atomized fat dystrophy was observed in six-month age animal liver after acute fipronil exposure. Changes were observed in periportal and centrolobular liver zones. Stellate cells of liver (Kupffer's cell) activation, circulatory disorders in the form of perisinusoidal space dilations were observed in liver parenchyma, but the beam structure of organ parenchyma remain preserved (Fig. 5).

Fatty degeneration was in centrolobular liver zones, fat drops were average sized in twelve-month age rats. Plethora of sinusoids was also revealed. However, the beam structure of organ was preserved (Fig. 6).

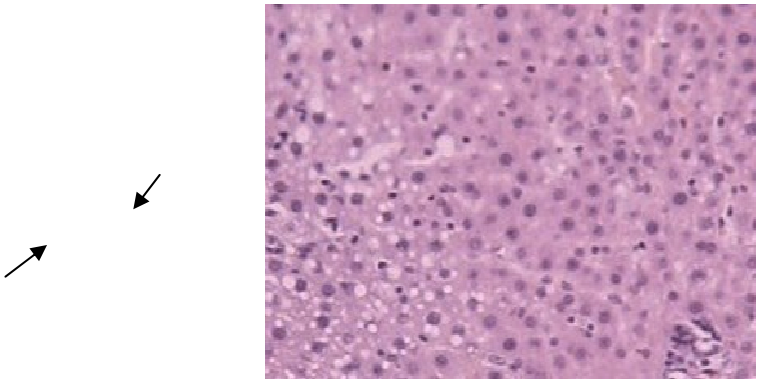


Fig. 4. Liver of 1 month age rats after fipronil acute exposure (x 200).

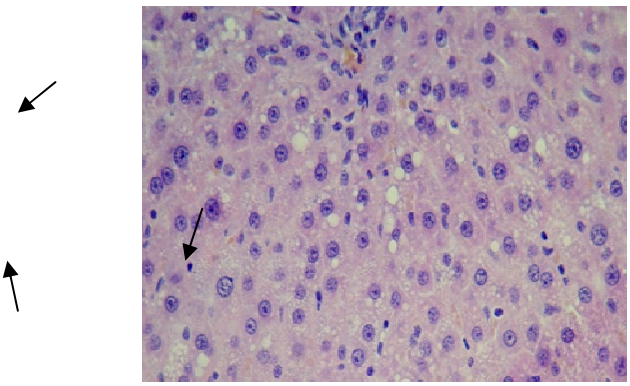


Fig. 5. Liver of 6 month age rats after fipronil acute exposure (x 200).

Fig. 6. Liver of 12 month age rats after fipronil acute exposure (x 200).

Significant structural changes of liver of different aged rats were observed after subacute fipronil exposure (Fig. 7, 8, 9). Progression of medium-dripping fat dystrophy in liver of one-month age rats was observed (Fig. 7). Multiple subacute fipronil exposure led to death of part of hepatocytes and development of inflammatory process. Lymphocytes, white blood cells, histiocytes were observed in centrolobilar and periportal liver zones, which evidenced of chronicle pattern of dystrophic and necrobiotic processes in liver after subacute xenobiotic exposure.

In six-month age rats the cytoplasm of separate hepatocytes was light with the central position of a nuclei, and cells looked dropsical (Fig. 8). The part of hepatocytes necrotized, that was shown by absence of nuclei in these cells. The structural changes of hepatocytes were diffusive, basically in centrolobular liver zones. Activation of liver stellate cells (number and size of Kupffer's cells was increased) occurred.

The large-dripping fat dystrophy was revealed in twelve-month age rat liver, whereas, in contrast to one- and six-month age rats after subacute xenobiotic exposure (Fig. 9). Large drops of fat and shearing of nuclei to periphery was observed almost in all parts of the liver. It is believed that fatty degeneration results in hepatocyte necrosis, with subsequent development of inflammatory processes. It is known, that at long-term xenobiotic exposure may lead to liver cirrhosis and animal death.

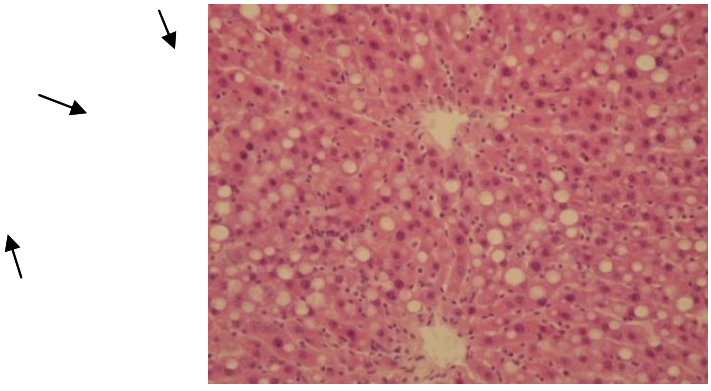


Fig. 7. Liver of 1 month age rats after fipronil subacute exposure (x 200).

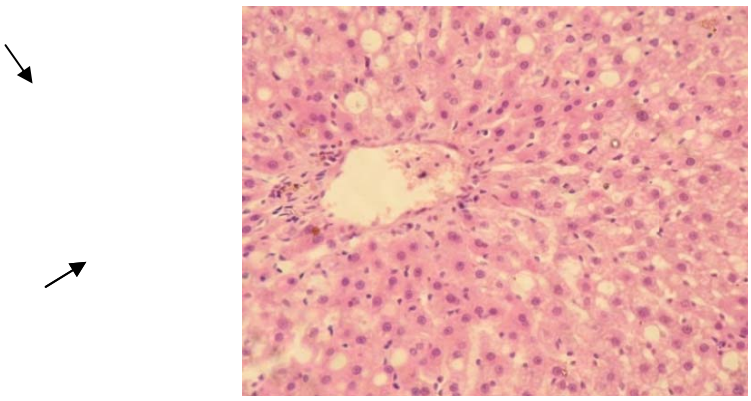


Fig. 8. Liver of 6 month age rats after fipronil subacute exposure (x 200).

It was determined the level of lipid peroxides and malonic dialdehyde in rats of different ages after fipronil exposure (Table 1). After acute fipronil exposure the content of lipid peroxides and malonic dialdehyde increased in comparison with the same parameters in intact rats, but that increase was not significant.

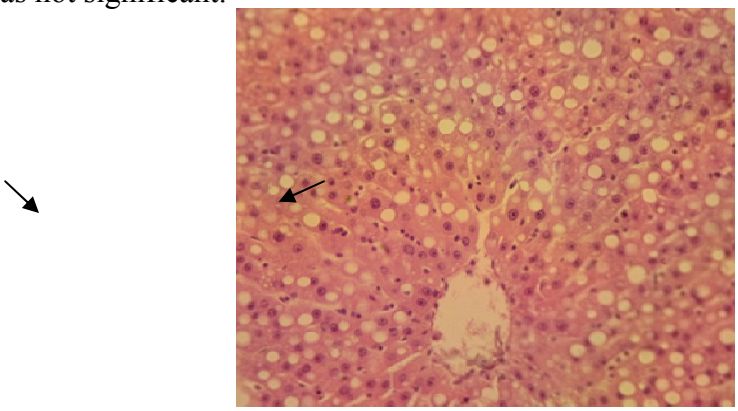


Fig. 9. Liver of 12 month age rats after fipronil subacute exposure (x 200).

After subacute fipronil exposure the significant increase of lipid peroxides and malonic dialdehyde level in livers of rats from different ages was observed, in comparison with control group. Comparative analysis of LPO rates between experimental groups determined the significant increase in LPO processes after subacute fipronil exposure, in comparison with rates after acute exposure. Lipid peroxides and malonic dialdehyde content increased with the increase of xenobiotic exposure time. Very high LPO level was demonstrated in 1-year old rats.

The content of cytochrome P450 in intact and fipronil exposed rat liver was determined (Table 2). The acute fipronil exposure caused the induction of cytochrome P450 synthesis in liver of experimental animals. Comparative analysis of cytochrome P450 content in animals from different age groups showed the least induction in 12-month old rats. But after subacute fipronil exposure the significant decrease of cytochrome P450 level was observed.

Above presented results testified, that fipronil at acute and subacute influence causes in a liver of all age groups a circulatory disorders, a fatty dystrophy and stellate cells of liver (Kupffer's cell) activation. It was noted, that the degree of everity of pathological changes increased with exposure time. As a rule result of a fatty dystrophy is the destruction of hepatocytes. The mass death of cells at long influence of xenobiotic can lead to a cirrhosis of a liver and a death of animals [14-17].

Table 1.

Level of lipid peroxides and malonic dialdehyde in rats liver tissues after fipronil exposure

Animal groups	Animal age (month)	Level of lipid peroxides (nm/g in liver)	Level of malonic dialdehyde

			(nm/g in liver)
intact	1	1.05 ± 0.31	1.43 ± 0.22
	6	0.85 ±0.24	1.04 ± 0.12
	12	0.96 ±0.51	1.14 ± 0.22
acute	1	1.67 ±0.36	1.80 ± 0.40
	6	1.21 ±0.17	1.30 ± 0.25
	12	1.85 ±0.24	2.07 ± 0.36
subacut e	1	4.04 ±0.18**	5.81 ± 0.45**
	6	1.95 ±0.25*	2.82 ± 0.30**
	12	5.41 ±0.62**	5.13 ± 0.30**
Note: * - P<0.01, ** - P<0.001 in comparison with control n=10			

Table 2.

The content of cytochrome P450
in intact and intoxic fipronil rat liver

Animal groups	Animal age (month)	Level of cytochrome P450 (nm/g in liver)
intact	1	5.71 ± 1.3
	6	7.76 ± 1.3
	12	6.63 ± 1.4
acute	1	41.25 ± 3.7***
	6	62.04 ± 5.2***
	12	27.21 ± 3.1***
subacute	1	28.53 ± 3.9***
	6	48.34 ± 7.4***
	12	18.43 ± 3.4*
*Note: * - P<0.01, ** - P<0.001 in comparison with control n=10*		

Accumulation of fat in hepatocytes can be also the result of formation toxic metabolities at biotransformation of pesticide. At long-term exposure of xenobiotic on animals the atomized fat dystrophy can pass into the large-dripping fat dystrophy, that conducts behind itself destruction of cells and, as consequence, decrease in function of a liver, and at generalized damages - even destructions of an organism.

There are not numerous data about cytotoxic effects of fipronil. So, in work A. Broadmeadow (1991) it is shown, that fipronil of 1.3 and 3.2 mg/kg caused in histostructural infringements in a liver of laboratory mice, expressed in a fatty dystrophy and necrotic processes in centrolobular liver zones , and also a follicular epithelial hypertrophy and vacuolization of the thyroid glands [18].

Fipronil was administered in the diet for four weeks to groups of five rats of each sex at concentrations of 0, 3.4, 6.9, 13, 24, or 45 mg/kg bw per day for males and 0, 3.5, 6.7, 13, 25, or 55 mg/kg bw per day for females. Although there were no clinical signs of toxicity, one female at 45 and 55 mg/kg bw died, however with no accompanying clinical or pathological findings. Body-weight loss or decreased body-weight gain seen in animals of each sex at doses > 13 mg/kg bw was temporary and possibly due to unpalatability, since food consumption was also decreased in these groups. The platelet counts of animals at 24 and 25 mg/kg bw, 45 and 55 mg/kg bw were marginally increased. The results of urinalysis were negative. Increased total protein and globulin were seen in all treated animals, and these increases were statistically significant; however, they were small in comparison with the values in controls and were poorly correlated with dose. Cholesterol levels were increased in females at all doses and in males at the high dose. The target organs were the liver and thyroid. Liver weights were significantly increased in females at all doses and in males at 24 and 25 mg/kg bw, 45 and 55 mg/kg bw . At necropsy, liver enlargement was observed in one or both sexes starting at 6.9 and 6.7 mg/kg bw

and five males and three females at 45 and 55 mg/kg bw had enlarged livers. Generalized hepatocyte enlargement was observed microscopically in one male at 13 mg/kg bw, with increasing incidence in animals of each sex at 24 and 25 mg/kg bw, 45 and 55 mg/kg bw. Thyroid follicular-cell hypertrophy, generally of minimal severity but of moderate severity in several males at 24 and 25 mg/kg bw, 45 and 55 mg/kg bw, was found in almost all treated animals but not in the controls. No NOAEL was identified because of changes in blood chemistry in one or both sexes, increased liver weights in females, and thyroid follicular-cell hypertrophy in animals of each sex at the lowest dose [19].

Lipid peroxidation with formation primary and secondary products at influence of the different chemical agents in organism can lead to infringement of a metabolism, causing serious destructive changes in organs and tissues. Probably, fipronil strengthens reactions free radical lipid oxidations, as is the main reason of structural infringements in a liver of intoxic animals. Increase of malonic dialdehyde demonstrates the progression of free radical reactions after fipronil toxic exposure. Obtained results coincide with histopathological data, indicating correlation between increase of fipronil toxic effects with increase of exposure time and animal age. One of the major enzymes of xenobiotic biotransformation system is the universal heme monooxygenaze – cytochrome P450. The acute fipronil exposure caused the induction of cytochrome P450 synthesis in liver of experimental animals, subacute fipronil exposure - significant decrease of cytochrome P450 level. Possibly, the decrease in detoxifying liver functions resulted dystrophic, necrobiotic processes in liver.

Conclusions

The investigation of liver structure from rats of different age groups after acute and subacute fipronil exposure allows to established, that given xenobiotic possesses marked hepatotoxic effects. Fatty degeneration, activation of stellate cells of liver (Kupffer's cell), the circulatory disorders in the form of perisinusoidal dilation, sinusoids plethora and hepatocytes necrosis were revealed in fipronil exposed animals. Hepatocytes of rats in age of 1 and 12 months were subjected to the most destructive changes. In addition, increased fipronil exposure resulted in increased degree of pathological changes severity and their spectrum. It was shown, that the content of lipid peroxides and malonic dialdehyde in liver of 1, 6, 12-month old rats after subacute exposure was significantly increased than in control group. It was established, that fipronil increased content of cytochrome P450, that is indicative of induction of xenobiotic biotransformation. The level of cytochrome P450 was significantly higher after subacute exposure comparing with control animlas, that determined LP activation and the progress of fatty degeneration and necrobiotic processes. The biochemical dates consistent with dates of histological investigation.

Acknowledgements

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