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Mechanism of action of *4-hydroxyphenylpyruvate dioxygenase* inhibitor herbicide on homoterm animals and humans

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Abstract

It was found that the main mechanisms of action of 4-hydroxyphenylpyruvatedioxygenase inhibitors are the development of tyrosinemia and alternations in thyroid hormone level as a result of hepatic enzyme induction.
The main target organs of its action are the eyes, liver and thyroid gland. It was proved that the most adequate model for extrapolation of the effects of tyrosinemia on humans are mice, because their tyrosine aminotransferase activity level is similar to that in humans.

Key words

4-hydroxyphenylpyruvatedioxygenase, tyrosinemia, tyrosine aminotransferase, target organs, corneal opacity.

INTRODUCTION

It is a well known fact that today nearly 1 billion people worldwide suffer from malnutrition. Over time, the situation can only become worse, considering that according to the UN and WHO data, the world's population will increase by more than 2 billion, and the demand for food – 70% by 2050 [1, 2]. The most effective way to combat hunger is to increase crop yields to provide the human population with sufficient vegetable foods, and livestock with fodder. The optimal and best cost-effective way of improving such a situation is to use crop protection chemicals at the present stage of agriculture development, technology, and existing areas of fertile lands.

However, at the same time, long-term epidemiological studies have found that pesticides, suppressing the body's defences and disrupting metabolism, promote the growth of population morbidity [3]. During the last decade, the attention of specialists in the field of preventive medicine worldwide has been attracted by the problem of increased incidence of endocrine diseases, including thyroid cancer, as a result of the adverse effects of chemical pollutants, particularly in areas affected by radioactive iodine, or endemic by iodine (as in many areas of Ukraine and Europe) [4, 5, 6]. In addition, there have been some reports of an increased incidence of thyroid pathologies, including cancer, among workers engaged in agricultural production and have had professional contact with pesticides [7, 8, 9]. The joint WHO and UN report (19 February 2013, Geneva) on the analysis of studies concerning the negative health effects of so-called endocrine disruptors, confirms the relevance of the presented study [4].

Objective. The aim of this study is an expert analysis of a new class of herbicides – 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibitors and mechanisms of their action, to assess the risk for humans, and to prevent acute and chronic poisoning and long-term effects, a precondition allowing the agricultural use of preparations based on these herbicides.

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RESULTS AND DISCUSSION

Hygienic classification. Information from websites and literature on the median lethal dose (LD_{50}) and concentration (LC_{50}) of the studied herbicides, their single-dose in the stomach (LD_{50} per os), when applied to the skin (LD_{50} per cutaneous) and inhalation (LC_{50} inhal) in rats [10, 14, 15, 16] is presented in Table 1. According to the hygienic classification of pesticides [13], based on the parameters of acute oral toxicity and dermal toxicity, all the examined herbicides are classified into toxicity class 4 (low-risk). The majority are moderately hazardous or toxic only by inhalation (Class 2 or 3); only pyrazoxyphen is extremely hazardous (Class 1). According to EPA US classification, benzofenap belongs to Class 4 of toxicity, isoxaflutole, pyrazoxyphen, tembotrion, topramezone – Class 3; according to the WHO classification, isoxaflutole, sulkotrione, tembotrion, topramezone belong to Class 3 and pyrazoxyphen is a hazard Class 2 substance. It should also be noted that pyrosulfotole, sulkotrione, tembotrion irritate mucous membranes and benzofenap, mesotrione, topramezon irritate the mucous membranes and skin [10, 16].

Toxicity of compounds. It was found that in subchronic and chronic experiments the magnitudes of subthreshold doses for male and female rats do not differ. However, the severity and range of symptoms in males are much larger, confirming their greater sensitivity to the negative impact of 4-HPPD inhibitors [17, 18]. In particular, changes in most of the studied parameters in females occur at dose levels which are one or two times higher than that of males. Some toxicologically significant effects in females are absent [17, 18].

It was also discovered in subchronic and chronic experiments that the major target organs under the action of

Table 1. General information on active ingredients and main toxicometric parameters

Chemical class	Trade name / IUPAC	Empirical formula	LD_{50} per os, mg/kg	LD_{50} per cut, mg/kg	LC_{50} inhal, mg/dm^3	ADI/Rfd, mg/kg
Benzoyl cyclohexanediones or triketones	Mesotrione / 2-(4-mesyl-2-nitrobenzoyl) cyclohexane-1,3-dione [10, 16]	$C_{14}H_{13}NO_7S$ [10, 16]	> 5,000 [10, 16]	> 2000 [10, 16]	4,75 [10, 16]	0,01 [10, 16]
	Sulkotrione / 2-(2-chloro-4-mesylbenzoyl) cyclohexane-1,3-dione [10, 16]	$C_{14}H_{13}ClO_5S$ [10, 16]	> 5,000 [10, 16]	> 4000 [10, 16]	>1,63 [10, 16]	0,0004 [10, 16]
	Tefuriltrione / 2-{2-chloro-4-mesyl-3-[(RS)-tetrahydro-2-furylmethoxymethyl]benzoyl} cyclohexane-1,3-dione [15]	$C_{20}H_{23}ClO_7S$ [15]	–	–	–	–
	Tembotrion / 2-{2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy) methyl] benzoyl} cyclohexane-1,3-dione [10, 16]	$C_{17}H_{16}ClF_3O_6S$ [10, 16]	> 2,500 [10, 16]	> 2,000 [10, 16]	>4.58 [10, 16]	0.01 / 0.01 [10, 16]
Benzoyl-pyrazoles	Benzofenap / 2-[4-(2,4-dichloro-m-toluoyl)-1,3-dimethylpyrazole-5-yloxy]-4′-methylacetophenone [10, 16]	$C_{22}H_{20}Cl_2N_2O_3$ [10, 16]	> 1,5000 [10, 16]	> 5,000 [10, 16]	1.93 [10, 16]	0.0015 [10, 16]
	Pyrosulfotole / (5-hydroxy-1,3- dimethylpyrazole-4-yl)(*a,a,a*-trifluoro-2-mesyl-*p*-tolyl)methanone[10, 16]	$C_{14}H_{13}F_3N_2O_4S$ [10, 16]	> 2,000 [10, 16]	> 2,000 [10, 16]	5.03 [10, 16]	0.01 / 0.2 [10, 16]
	Pyrazoxyphen / 2-[4-(2,4-dichlorobenzoyl)-1,3-dimethylpyrazole-5-yloxy]acetophenone [10, 16]	$C_{20}H_{16}C_{l2}N_2O_3$ [10, 16]	>5,000 [10, 16]	–	0.28 [10, 16]	–
	Pyrazolinate / 4-(2,4- dichlorobenzoyl)-1,3-dimethylpyrazole-5-yl toluene-4-sulphonate [14]	$C_{19}H_{16}C_{l2}N_2O_4S$ [14]	9,550 [14]	>5,000 [14]	2.5 [14]	0.006* [14]
	Topramezone / [3-(4,5-dihydro-1,2-oxazole-3-yl)-4-mesyl-*o*-tolyl](5-hydroxy-1-methylpyrazole-4-yl) methanone [10, 16]	$C_{16}H_{17}N_3O_5S$ [10, 16]	> 2,000 [10, 16]	> 2000 [10, 16]	>5,4 [10, 16]	0.0008 / 0.001 [10, 16]
Oxazoles	Isoxaflutole / (5-cyclopropyl-1,2-oxazole-4-yl)(*a,a,a*-trifluoro-2-mesyl-*p*-tolyl) methanone [10, 16]	$C_{15}H_{12}F_3NO_4S$ [10, 16]	> 5,000 [10, 16]	> 2000 [10, 16]	5.23 [10, 16]	0.02 [10, 16]
Unclassified active ingredients or bicyclooctene compounds	Benzobicyclon / 3-(2-chloro-4-mesylbenzoyl)-2-phenylthiobicyclo [3.2.1]octo-2-ene-4-one [10, 16]	$C_{22}H_{19}ClO_4S_2$ [10, 16]	> 5,000 [10, 16]	> 2000 [10, 16]	2.72 [10, 16]	–
	Bicyclopirone / 4- hydroxy -3-{2-[(2-methoxyetoxy) methyl]-6-(trifluoromethyl)-3-pyridylcarbonile} bicyclo[3.2.1]octo-3-ene-2-one[10, 16]	$C_{19}H_{20}F_3NO_5$ [10, 16]	980 [10, 16]	–	–	–

* – ADI value was established in Japan

4-HPPD inhibitors are the liver (hepatocellular hypertrophy in rats and mice), thyroid gland (follicular cell hypertrophy in rats and dogs), and the eye (corneal opacity and chronic keratitis in rats) [17, 18].

Mode of action. Activity of 4-HPPD inhibitors is conditioned by the presence of chelating diketone moiety and aryl substituent in the structure of molecules (Tab. 2, 3). The active centre of the 4-HPPD enzyme is formed by two histidine and glutamic aminoacid residues that hold catalytic cation of iron (II). There are also two phenylalanine residues responsible for hydrophobic interactions which are part of the active site of the enzyme. The 4-HPPD inhibitors bind to the enzyme by interaction between chelating diketone moiety and iron cation. At the same time, aryl substituent of the inhibitor interacts with two phenylalanine residues. Variation of radicals in aryl substituent causes the specific physical and chemical characteristics of inhibitors. It should be noted that in the liquid biological matrices, the chelating inhibitor fragment exists in tautomeric forms of diketone and keto-enol.

The common structural feature for 4-HPPD inhibitors is the presence of an aryl fragment included in 2,4- and 2,3,4-substituted benzoyl group. The structural difference consists in a different way of chelating moiety forming during the combination of the substituted benzoyl group with the cyclohexanedione ring (benzoylcyclohexanediones) or heterocycle (benzoylpyrazoles and oxazoles), or bicyclooctene group (benzobicyclon). Bicyclopirone, like benzobicyclon, contains the bicyclooctene group which is connected to the heteroaroyl group (Tab. 2, 3).

It should be noted that pyrazoxyfen and pyrazolinate have a less pronounced capacity to inhibit 4-HPPD activity. This effect becomes more pronounced when they are transformed in water or plants into metabolite 4-(2,4-dichlorbenzoyl)-1,3-dimethyl-5-hydroxypyrazole by hydrolysis of shifted 5-hydroxypyrazole group (O-R^5→O-H) [19]. This metabolite containing a chelating fragment that binds more effectively with the active centre of the enzyme, thereby blocking it. Perhaps this tendency is inherent in benzofenap that has substituted the 5-hydroxypyrazole group. The activity of isoxaflutole is also related to its metabolite – a corresponding dyketonitrile formed in the oxazole heterocycle splitting *in vivo* [20].

Sensitivity of animals. As a result of data analysis of subchronic and chronic experiments, it was found that the toxicity of mesotrione, topramezon, isoxaflutole, pyrosulfotole, sulkotrione and tembotrion, after repeated exposure, depends on the type of animal: rats are the most sensitive, followed by mice and/or dogs [17, 18, 21]. Thus, calculated by the toxic effects, the species sensitivity factor

Table 2. Chemical structure of benzoyl cyclohexanediones and benzoyl-pyrazoles

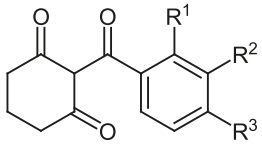
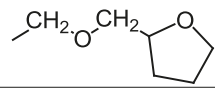
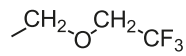
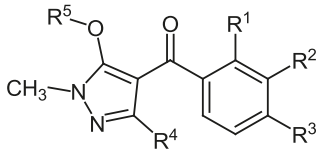
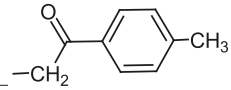
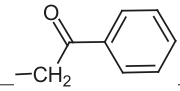
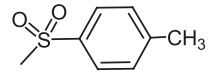
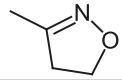
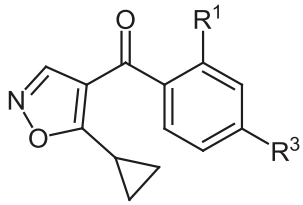
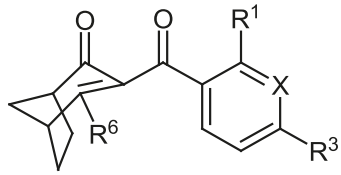
Chemical class	Structural formula	Substance	R^1	R^2	R^3	R^4	R^5
Benzoyl cyclohexanediones (triketones)		Mesotrione	NO_2	H	SO_2CH_3	-	-
		Sulkotrione	Cl	H	SO_2CH_3	-	-
		Tefuriltrione	Cl		SO_2CH_3	-	-
		Tembotrion	Cl		SO_2CH_3	-	-
Benzoyl-pyrazoles		Benzofenap	Cl	CH_3	Cl	CH_3	
		Pyrazoxyphen	Cl	H	Cl	CH_3	
		Pyrazolinate	Cl	H	Cl	CH_3	
		Pyrosulfotole	SO_2CH_3	H	CF_3	CH_3	H
		Topramezone	CH_3		SO_2CH_3	H	H

Table 3. Chemical structure of oxazoles and bicyclooctene compounds

Chemical class	Structural formula	Substance	R^1	R^3	R^6	X
Oxazoles		Isoxaflutole	SO_2CH_3	CF_3	-	-
Unclassified active ingredients or bicyclooctene compounds		Benzobicyclon	Cl	SO_2CH_3	SPh	CH
		Bicyclopirone	$CH_2OCH_2CH_2OCH_3$	CF_3	OH	N

(SSF) for mesotrione in subhronic experiments in male rats is 800.0, female rats – 266.7; male mice – 16.7, female mice – 1.8, compared to the most resistant species – dogs [22]; SSF for topramezon in male rats is – 2080.9, female rats – 162.4; male dogs – 12.6, female dogs – 2.0, compared to the most resistant species – mice [23]. SSF for topramezon, according to the results in chronic experiments in male rats is 47.5, female rats – 52.0; male dogs – 6.6, female dogs – 1.7, compared with mice [23]. Based on information about the NOAEL values in subchronic and chronic experiments (Tab. 4), we also calculated SSF for 4 studied herbicides (Tab. 5), where the species most susceptible to its action were rats.

Expressed animal species sensitivity to the action of 4-HPPD inhibitors is related to the mechanism of its effects and peculiarities of tyrosine metabolism in different mammals species [15, 18, 21, 24]. The severity of tyrosinemia

Table 4. Values of NOAEL for studied herbicides – 4-HPPD inhibitors

Name of substance	NOAEL values (mg/kg) depending on the duration of the experiment and animal species					
	Subchronic experiment			Chronic experiment		
	90 days			24 months, rats	18 months, mice	12 months, dogs
	rats	mice	dogs			
Isoxaflutole	3 [10]	–	1,000 [10]	2 [10]	♂ 3.2 [10] ♀ 4.0 [10]	♂ 44.81 [10] ♀ 45.33 [10]
Pyrosulfotole	♂ 66 [10] ♀ 2,32 [10]	♂ 259 [10] ♀ 326 [10]	♂ 40 [10] ♀ 33 [10]	♂ 1.0 [10] ♀ 1.4 [10]	♂ 13.6 [10] ♀ 16.7 [10] (LOAEL)	♂ 7 [10] ♀ 33 [10]
Sulkotrione	3,3 [10]	–	40 [10]	0.04 (LOAEL) [10]	♂ 332 [10] ♀ 5.2 [10]	50 [10]
Tembotrion	♂ 0,09 [17] ♀ 0,07 [17]	♂ 64.0 [17] ♀ 75.6 [17]	♂ 26.7 [17] ♀ 28.5 [17]	0.04 [17]	♂ 4 [17] ♀ 5 [17] (LOAEL)	♀ 10.2 [17] (NOAEL) ♂ 2.5 [17] ♀ 41.6 [17] (LOAEL)

Table 5. Variability of animal species sensitivity to the influence of studied herbicides – 4-HPPD inhibitors

Name of substance	Animal species	SSF depending on the duration of the experiment and animal species			
		Subchronic experiment		Chronic experiment	
		males	females	males	females
Isoxaflutole	mice/rats	–	–	1.6	2.0
	dogs/rats	333.3		22.1	22.7
	dogs/mice	-		14	11.3
Pyrosulfotole	mice/rats	3.9	140.5	13.6[2]	11.9[2]
	dogs/rats	0.6	13.1	7.0	23.6
	mice/dogs	6.5	9.9	1.9[2]	2.0[2]
Sulkotrione	mice/rats	–	–	8,300.0[3]	130.0[3]
	dogs/rats	12.1		1250.0[3]	
	mice/dogs			6.6	9.6
Tembotrion	mice/rats	711.1	1,080	100.0[2]	125.0[2]
	dogs/rats	296.7	407.1	–	255.0
				62.5[1]	1,040.0[1]
	mice/dogs	2.4	2.7	1.6[1,2]	0.1[1,2]

1 – LOAEL value was substantiated in chronic experiment for dogs;
2 – LOAEL value was substantiated in chronic experiment for mice;
3 – LOAEL value was substantiated in chronic experiment for rats.

provoked by 4-HPPD inhibitors depends on the tyrosine aminotransferase (TAT) activity which in mice is 3–5 times higher, and the level of tyrosinemia is lower than in rats (Tab. 6).

Table 6. Activity of enzymes and maximum tyrosine concentration in blood serum [18]

Species	Gender	Basal enzymes activity, μg of oxygen /min/mg of protein		Maximum tyrosine concentration, nmol/ml
		4-HPPD	TAT	
Rats	♂	0.27	1.7	3,000
	♀	2.4	3.4	1,500
Mice	♂	0.07	7.8	800
	♀	0.15	10.5	800
Humans	♂, ♀	-	4.5–7.3	800–1,200

Mice are therefore more resistant to the actions of 4-HPPD inhibitors and the development of tyrosine-induced toxicity in these animals is much less evident. In addition, resistance of the females to the action of 4-HPPD inhibitors is greater, especially with respect to tyrosine-induced symptoms, which may be due to higher TAT activity (in rats – almost 200% and in mice – 35%), compared to males (Tab. 6).

Ways of tyrosine metabolism and types of tyrosinemia. There are three ways of tyrosine metabolism in mammals: 1) in the liver – converting it into 4-hydroxyphenylpiruvat (4-HPP) with the help of TAT, followed by decarboxylation to homogentisate which, in turn, then converts into acetylacetate and fumarate; 2) in the nervous tissue – conversion using tyrosinehydroxylase to 3,4-dihydroxyphenylalanin (DOPA) and conversion to dopamine by dopa-decarboxylase participation, formation of norepinephrine and epinephrine; 3) in melanocytes – dopaquinone is formed from DOPA which is then spontaneously converted to melanin [26]. The examined class of pesticides affects the first pathway of tyrosine metabolism. Figure 1 presents the mechanism of 4-HPPD inhibitors action and possible tyrosinemia development.

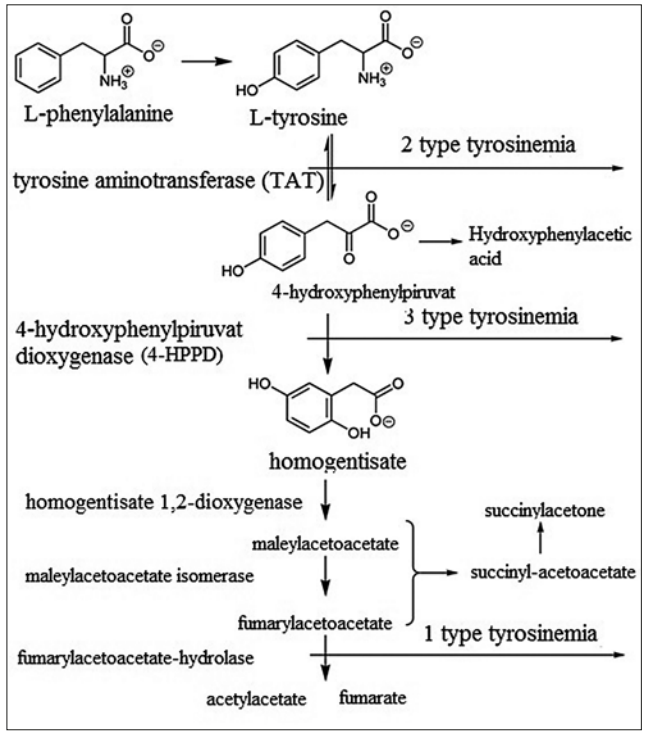


Figure 1. Mechanism of 4-HPPD inhibitors action and possible ways of tyrosinemia development

At the inhibition of 4-HPPD action, TAT becomes the main enzyme catalyzing the conversion of tyrosine. However, in rats, especially males, the activity of this enzyme is insufficient for tyrosinemia occurrence and to maintain the level of tyrosine which would be below toxic level. TAT is the first and dose-dependent enzyme in the cascade of tyrosine conversion into 4-HPP, which then converts into homogentisic acid with the help of 4-HPPD. If this pathway is limited due to the inhibition of 4-HPPD, its substrate 4-HPP is excreted in the urine directly, or turned into other phenolic acids (e.g. *p*-hydroxyphenylacetic acid), before excretion with the urine. Since the reaction involving TAT is reversible, the 4-HPP may again convert into tyrosine. Severe tyrosinemia in rats leads to the occurrence of so-called critical effects – eye damage (Fig. 2).

In contrast, in mice, 4-HPPD inhibition develops to a much lesser degree due to the higher basal TAT activity and, consequently, much lower tyrosinemia, that does not lead to the occurrence of critical effects. TAT activity in mice and in humans is at the same level, but is much higher than in rats, suggesting that humans will not develop such a severe tyrosinemia as rats. In addition, tyrosinemia in male rats is more pronounced (3,000 nmol/ml) than in females (1,500 nmol/ml). It should be noted that tyrosinemia in mice can reach only 800 nmol/ml, but even at these parameters critical changes in the eyes of these animals do not occur. In experiments on rats and mice, it was found that the tyrosinemia threshold for the appearance of changes in eyes is 1,000 nmol/ml [18, 27].

The described mechanism of development is typical of type III tyrosinemia. This type of tyrosinemia (due to lack of 4-HPPD) is rarely recorded among humans and can be recorded only in newborns, mostly premature, as a transitional neonatal disorder of unknown etiology. Pathology of the nervous system and minor mental retardation was observed in patients in this state, but special studies revealed no relationship between the level of tyrosinemia and clinical symptoms. Moreover, the pathology of the nervous system has not been observed in experiments studying the reproductive and embryotoxicity in rats [28].

Three types of tyrosinemia are described in humans. The most severe tyrosinemia is characteristic for a rare congenital metabolic disorder caused by TAT deficiency. This is type II tyrosinemia or Richner-Hanhart syndrome (oculocutaneous tyrosinemia, or skin-eye syndrome), which is inherited in an autosomal recessive pattern. Lack of TAT leads to an increase in levels of tyrosine in blood – up to 3,300 nmol/l, crystallization of accumulated tyrosine in the epidermis (in the form of painful hand-foot hyperkeratosis), and in the cornea (as herpetiformic ulcers), often with mental retardation. Patients with such defects have a very low tyrosine clearance, and a diet excluding tyrosine and phenylalanine intake can reduce tyrosine levels below 800 nmol/l (the threshold damage to the eye is reversible) [29].

Type I tyrosinemia (hepatorenal tyrosinemia) is another inherited fatal disease resulting from fumarylacetoacetate hydrolase enzyme deficiency, which is also an autosomal recessive trait. Disorder of tyrosine catabolism at this stage leads to accumulation of maleylacetoacetate, fumarylacetoacetate and its metabolites which, in turn, lead to diseases of the liver (hepatic failure, cirrhosis) and kidney (renal tubular dysfunction with the development of hypophosphatemic rickets, sometimes – renal insufficiency). Treatment by tyrosine-free and phenylalanine-free diet does not prevent a fatal outcome. The only effective treatment today is liver transplantation. However, the use of (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) (NTBC), which is actually a chemical analogue of mesotrione, prevents the occurrence of hepato- and nephrotoxic metabolites by irreversible inhibition of 4-HPPD. For example, 200 children with tyrosinemia of a specified type received NTBC to reduce 4-HPPD activity to a minimum. The concentration of tyrosine in the blood was below 800 nmol/ml, and eye damage occurred in only 5 cases, and therefore not considered as proof of its connection with the use of NTBC. At the same time, inhibition of 4-HPPD was complete in 10 healthy adult male volunteers when administered NTBC at 1 mg/kg, and the level of tyrosinemia reached 1200 nmol/ml, followed by a decline to 800 nmol/ml. This level of tyrosine lasted for 2 weeks before taking the next dose of NTBC. None of the men had signs or symptoms of substance toxic effects [18, 27].

Peculiarities of metabolism in human organism. To confirm that a human under the action of 4-HPPD inhibitors will not develop severe tyrosinemia (when it reaches a threshold of 800 nmol/ml), studies should be conducted on volunteers concerning the effects of tyrosine and mesotrione and sulkotrione on metabolism, as the most commonly and continuously used substances [18, 27]. Thus, the effect of oral administration of a single dose of mesotrione at a level of 0.1; 1.5 and 4.0 mg/kg was studied in the volunteers. The peak of tyrosinemia was observed 12 hours after administration. Maximum concentration of tyrosine was 300 nmol/ml when administered at a dose of 4.0 mg/kg (an almost 6-fold increase compared to the initial dose), but after 48 hours it returned to the reference level. Most of the received mesotrione was quickly excreted with urine [18].

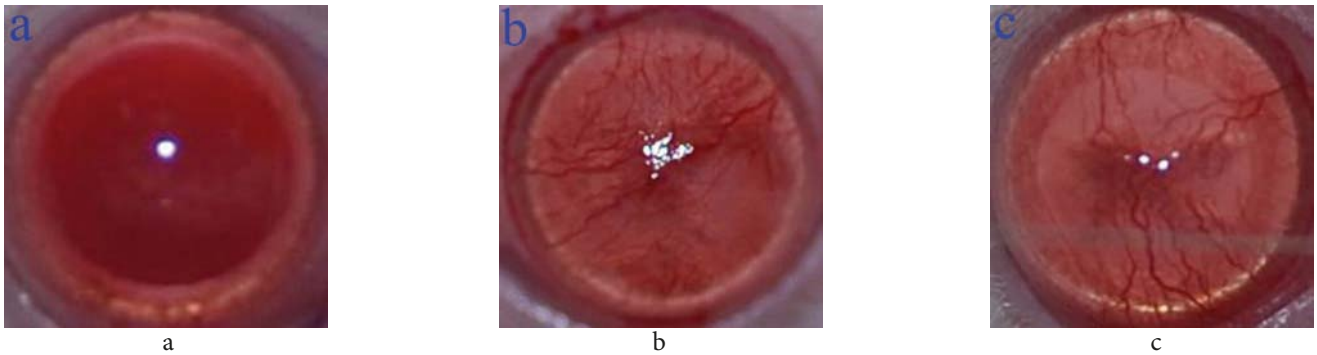


Figure 2. Changes in the eye of rats under 4-HPPD inhibitors action [29]:
a – normal fundus of eye of control animals;
b, c – changes in the eyes of experimental rats.

Similar studies were conducted with sulkotrione [27]. A total of 145 blood samples were collected from exposed and control volunteers. The concentration of tyrosine in the blood of exposed volunteers was 94.14±19.67 nmol/ml for men (n – 37) and 98.85±21.66 nmol/ml for women (n – 31). Whereas in the control group, the values were 97.60±16.27 and 100.2±18.40 nmol/ml for men (n – 35) and women (n – 35), respectively. Tyrosine concentration in the blood was 82.02±23.26 nmol/ml in the laboratory staff who had contact with sulkotrione (7 men and women); and 90.36±19.27 nmol/ml in those who were not in contact (10 men and women) [27]. It was found that sulkotrione does not lead to a significant increase in tyrosine in human blood, although the animals exposed to its effect showed severe tyrosinemia.

Further evidence for the greater resistance of the human body to the action of 4-HPPD inhibitors is shown by the poisoning of two men, aged 30 and 37 years, who took sulkotrione with the intention of committing suicide [30]. Apart from increased sulkotrione concentrations in blood, the only symptom of poisoning was vomiting. Only one of the men developed tyrosinemia which, however, was not accompanied by any pathological symptoms in the eyes. In the other patient, transient hypotension and renal dysfunction were detected, but eye abnormalities were absent. Both cases ended positively, and the patients survived.

CONCLUSION

These arguments suggest that the extrapolation of tyrosinemia caused by 4-HPPD inhibitors in rats to humans is not justified. Thus, in all publications cited it was shown that the mice are a more appropriate model for studying the toxic effects resulting in tyrosinemia, because TAT activity is similar to that in humans. Rats, by contrast, are not an acceptable model due to the low activity of TAT. Therefore, despite the very low probability of the occurrence of tyrosinemia-caused pathological changes, including eye damage in humans, this new class of herbicides 4-HPPD inhibitors requires detailed study and the regulation of its application in agriculture in Ukraine and worldwide.

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