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HYGIENIC ASSESSMENT OF TOXICODYNAMICS PECULIARITIES AND MECHANISM OF ACTION OF OXYACETAMIDE CLASS HERBICIDE (FLUFENACET) ON HOMOIOTHERMS AND HUMAN BODY

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ABSTRACT. *Analysis of literature data regarding toxic properties of a new oxyacetamide class herbicide, flufenacet, in acute, subchronic, and chronic experiments among different species of animals was performed. It was established that according to the hygienic classification of pesticides (DSanPin (State sanitary norms and rules) 8.8.1.002-98), flufenacet belongs to the class 2 of hazard (hazardous). It was shown that the primary target organ for flufenacet is the liver, and secondary one is the thyroid gland. As a result of the conducted analysis, acceptable daily intake of flufenacet for human at the level of 0,004 mg/kg was justified.*
Key words: *herbicides, oxyacetamides, toxicity, acceptable daily intake.*

Relevance. Pesticides are essential components of agricultural production management. In Ukraine, the range of pesticide drugs annually increases. It may cause pollution of the environmental objects and change population health [1, 2]. The first place in the structure of the range of pesticides approved for use in Ukraine belongs to herbicides of different classes [3] that differ in toxicological properties.

In Ukraine, according to the current approaches, an obligatory condition for the registration of new pesticides is their complete toxicological and hygienic assessment, as well as justification of acceptable daily intake (ADI) for human [4]. ADI is the main parameter of substance toxicity that is used for complex assessment of total penetration of pesticides to the human body by different ways [5, 6].

One of the representatives of new modern herbicides is flufenacet — active substance of the preparation Artist, WG. Flufenacet is a herbicide that inhibits enzyme elongase of the long-chain fatty acids in plants. Mechanism of flufenacet action on the harmful plants is the same as the action of chloroacetanilides and is typical for herbicides [7].

Objective of the work was a hygienic assessment of hazard according to toxicological criteria of a new herbicide, flufenacet, scientific justification of its acceptable daily dose.

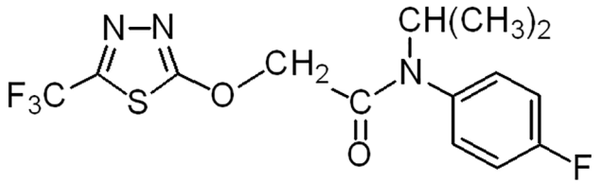
Materials and methods. This work presents a hygienic assessment of flufenacet based on the expert and analytical exploration of US EPA, FAO/WHO, Health Canada data [8–11] regarding the results of toxicological experiments of different duration. Flufenacet belongs to the chemical class of oxyacetamides. The main physical and chemical properties of flufenacet are provided in Table 1.

Assessment of flufenacet toxicity for laboratory homoiotherms and their hazard for human was performed according to the literature and Internet data in compliance with the current Ukrainian hygienic classification of pesticides by the degree of hazard (DSanPin 8.8.1.002-98) [12]. Acceptable daily intake (ADI) of the study substance for human was justified according to the methodological complex hygienic rating of pesticides using technical approaches set out therein [4, 5].

Results. According to the results of the analysis and synthesis of literature data and web-sites [8–11] regarding primary toxicological assessment of flufenacet (Table 2), it was established that according to hygienic classification of pesticides by the hazard degree, flufenacet belongs to class 3 of hazard (moderately hazardous) according to the parameters of acute oral toxicity, according to percutaneous toxicity— class 4 (low-hazard), according to acute inhalation toxicity — to class 2 (hazardous), according to skin irritation — to class 4 (has no irritant effect), according to mucous membrane toxicity — to class 3 (has a mild irritant effect). The study substance has no allergenic properties in the test on guinea pigs.

Table 1

Physicochemical properties of flufenacet [8]

Parameter	Value
Chemical name (IUPAC)	4'-fluoro-N-isopropyl-2-[5-(trifluoromethyl)-1,3,4-tridiazole-2-yloxy]acetanilide
CAS №	142459-58-3
Element formula	$C_{14}H_{13}F_4N_3O_2S$
Relative molecular weight	363,3
Structural formula:	
Steam pressure, mPa (20 °C)	9×10^{-2}
Solubility in water, mg/dm^3 (25 °C)	56,0
Solubility in organic solvents, g/dm^3 (20 °C)	n-hexane — 8.7, toluene, dichloromethane, acetone, dimethyl formamide, acetonitrile, dimethyl sulfoxide – > 200, 2-propanol — 170, polyethylene glycol — 74
Distribution factor n-octanol/water (log Ko/w) (24 °C)	3,2
Melting point, °C	76-79

According to our data [11], flufenacet has a low toxicity for mice, moderate toxicity for rats, no toxicity upon the exposure to skin of rabbits in studied maximum concentrations, it minimally irritates mucosa of eyes, and does not irritate skin, allergen. In short-term and chronic studies in mice, dogs, and rats, the same effects were observed.

Table 3 provides values of the non-acting doses, established in subacute, subchronic, chronic experiments according to the data of open sources [9–11]. In a subacute experiment in rats, no clinical symptoms of general toxic action were observed [9–11]. Based on the increase of liver weight, hypertrophy of the cells, and the level of T4, it was established that flufenacet acts as the inductor of smooth endoplasmatic reticulum of hepatocytes and function of oxidation of cytochrome P450 by phenobarbital type. According to [9–11], recommended NOAEL is 1,000 mg/kg. We think, NOAEL in this study is 150 mg/kg.

In a subchronic experiment in rats, substance in concentration of 3,000 ppm led to the body weight loss. Established NOEL was 7,2 mg/kg (100 ppm) [9–11]. Since males receiving the substance in concentration of 100 ppm showed reduced thyroxine level, this dose was higher than NOEL (probably, it may be considered as NOAEL). In subchronic experiments in rats, the substance in concentration of 60 and 100 ppm in females led to the statistically significant reduction of thyroxine level [9–11]. Based on the obtained results, accepted NOEL in the subchronic experiment is 1,7 mg/kg (30 ppm).

In rats that received the substance in maximal doses for 2 years , the increase of the incidence of hepatocytomegalia, necrosis of isolated hepatocytes, miliary hyperplasia/fibrosis, cataract, pigmentation of the spleen, increased incidence of hyperplasia of renal pelvises, cystic hyperplasia of endometrium, granulomatous pneumonia were noticed. The most sig-

Table 2

Parameters of acute toxicity of flufenacet [8-10]

Experiment, species of animals, toxicometry parameters, measuring units	Value of the parameter for substance	Class of hazard (DSanPin 8.8.1.002-98)
Acute oral toxicity, rats LD_{50}, mg/kg	589 (♀) 1 617 (♂)	3
Acute percutaneous toxicity, rats LD_{50}, mg/kg	>2 000 (♀♂)	4
Acute inhalation toxicity, rats, LD_{50}, mg/m^3	> 3740 (♀♂)	2
Irritation action on mucous membranes, rabbits	none	4
Irritation action on skin, rabbits	poor	3
Sensitizing action, guinea pigs	none	4

Table 3

The value of inactive doses of flufenacet in subchronic and chronic experiments after oral administration [9–11]

Nature of action	Species of animals, duration	Dose, ppm	NO(A)EL, mg/kg
Subchronic toxicity	rats, (90 days)	0, 15, 30, 60 and 100	1,7 (30 ppm)
	mice, (90 days)	0, 100, 400, 1600 and 4000	100 ppm (18.3 mg/kg for ♂ and 24.5 mg/kg for ♀)
Chronic toxicity	rats (2 years)	0, 25, 400 и 800	1,2
	mice (2 years)	0, 50, 200 и 400	50 ppm (♂ — 7.4 mg/kg, ♀ — 9.4 mg/kg)
	dogs (1 year)	0, 40, 800 и 1600	40 ppm (1.29 mg/kg for ♂ and 1.14 mg/kg for ♀)

Notes: 1. NOEL — no effect level; dose upon which no effects are observed;
2. NOEL — no adverse effect level; dose upon which no damaging effects are observed.

nificant from toxicological point of view is the increase of methaemoglobin content. NOEL was established at the level of 25 ppm [9–11].

In a chronic experiment in CD-1 mice, corneal opacity occurred [11]. During histological examination, cataract was found in animals that received the substance in the doses of 50 ppm and higher. A dose of 50 ppm [10,11] may be considered as inactive dose, considering changes in the content of haemoglobin, however, by cataractogenic effect of this dose is higher than NOEL.

In a chronic experiment in Beagles it was established that in maximal doses the substance caused abnormalities in behaviour (hyporeactivity, reduced response to movements and sounds, hyperreactivity/hypertension, abnormal body position, impaired walking, optic nystagmus/strabismus) [11]. It was established that dogs are the most sensitive to the action of flufenacet. Since in the brain of the dog, the main metabolite of substance — tiadon — was found in significant amount, it is considered that this metabolite easily pene-

trates blood-brain barrier, and detected changes in the dog body are the result of tiadon effect [9–11].

In chronic experiments in all three species of animals (mice, rats, dogs), the same deviations associated with flufenacet action were found in the following organs: kidneys, bone marrow, spleen, thyroid gland [9–11]. Changes in eyes: cataract (mice and rats), scleral mineralization (rats), vacuolization of the epithelium of ciliary and body and cystic vacuolization of the peripheral part of the iris (dogs) [11]. Also dogs and rats receiving maximal doses of the substance showed axonal oedema of the brain and spinal cord [11]. Table 4 summarises the results regarding delayed effects of flufenacet action.

Mutagenic activity of the substance was studied on the sufficient number of tests (Ames test, induction test for gene mutations, test for unscheduled DNA synthesis in vitro, the test for chromosomal abberations, micronuclear in vivo test) [8–11]. Analysis of the results suggest the lack of mutagenic activity in the study substance. No carcinogenic activity of flufenacet was found in the experiments in rats and mice [8–11].

Clinical signs of the neurotoxic effect of flufenacet were observed in acute and subacute experiments [10, 11]. In acute experiments in rodents, disorders in coordination and walking, reduced activity were found, in subacute — reduction of the strength of forepaws, disorders of coordination of righting reflex, decrease of the body temperature [11]. And dogs in the same studies showed only head bending at the end of 1-year experiment [11]. Also, in 1-year experiment in dogs, non-linearity of the excretion of the main flufenacet metabolite — tiadon was found suggesting the depletion of metabolic processes upon high doses [11].

For more detailed study of the effect of the main metabolite tiadon, additional study was performed in dogs [11], where axonal oedema of the brain and spinal cord, as well as the reduction of activity of glutathione reductase of the brain stem and cerebellum upon high doses were observed, confirming the hypothesis that limitation of glutathione-dependent metabolic pathways and the increase of antioxidative stress is a reason for the described changes in the brain [11]. In the experiments that explored toxicokinetics of tiadon, it was detected in the cerebral extractions [11]. It is known that inhibition of glutathione-dependent metabolic pathways by 20 % leads to the damage of cells that significantly depends on the level of oxygen leading to their apoptosis [11, 13]. The above data together suggest that the detected neurotoxic effects are associated with increased tissue disposal of glutathione that leads to the reduced protection of cells from oxidative stress.

Studies of the effect of flufenacet on the reproductive function were performed in rats in the test system of two generations [9–11]. No hazardous effect on the offspring was found [9–11]. NOEL according to the reproductive parameters — 500 ppm, by systemic toxicity — 20 ppm, considering the increase in the liver weight and the presence of hepatocellulary hypertrophy in females of generation F_1 at 100 ppm.

Table 4

Delayed effects of flufenacet action [9-11]

Nature of action	Species of animals	Doses, ppm	NO(A)EL, mg/kg
Carcinogenic activity	rats (2 years)	0, 25, 400 i 800 ppm	800 ppm
	mice (2 years)	0, 50, 200 and 400 ppm	400 ppm
Reproductive toxicity	rats (test in two generations)	0, 20, 100 i 500 ppm	by reproductive parameter — 500 ppm; by systemic toxicity — 20 ppm
Embryotoxicity	rats	0, 5, 25 and 125 mg/kg	25 (by toxicity for mothers and by embryotoxicity)
	Rabbits	0, 5, 25, 125 and 200 mg/kg	by maternal toxicity — 5, by embryotoxicity — 25.

Embryotoxic and teratogenic action was studied in rats and rabbits [9—11]. It was established that the substance has no effect on the level of mortality and the development of clinical symptoms of general toxic action in experiments in rats [9–11]. In foetus, upon the action of maximal dose, slight body weight loss, increase in the incidence of delayed ossification in some animals and/or increase of the rate of additional ribs were observed [9–11]. Teratogenic effect was not observed.

In the experiments in rats, upon exploration of embryotoxicity and teratogenic effect of the substance in two maximal doses in females, the reduction of body weight gain during priming, histological changes in the liver were observed [9–11]. In foetuses, upon the action of maximal dose, the reduction of foetus body weight and increase of the incidence of delayed ossification of skeletal bones was observed [9–11]. Teratogenic effect was not observed.

In the range of experiments in rats, it was registered that flufenacet induces liver metabolism (proliferation of endoplasmatic reticulum, hepatocellulary hypertrophy, increase of liver weight), and also reduced blood T4 level [10, 11]. There are literature data [14-16] that the range of active substances of pesticides reduce the level of hormones of the thyroid gland, without direct action on it, due to the induction of liver metabolism (i. e. inductors of liver metabolism by phenobarbital type).

In order to clarify this issue regarding flufenacet, studies were performed in rats [11] that were thyroidectomised and received replacement hormonal therapy through implanted minipump during 4 weeks in the doses of T3 and T4 that corresponded to the euthyroid control [11]. In 7 days after implantation of minipump in control and experimental animals, they began to receive flufenacet with feed. Furthermore, they studied functions of thyroid gland exposed to flufenacet via the reduction of changes in iodine consumption [11]. The reduction of T4 level and liver enlargement was observed both in the control and in the experimental animals, although functional condition of the thyroid gland measured by iodine consumption by it was unchanged [11]. Therefore, it was established that flufenacet has no effect on hormone synthesis of the thyroid gland.

Also experiments that studied metabolism of tyrosine in the liver were conducted [10, 11]. These experiments showed a significant increase of the activity of hepatic uridineglucuronyl transferase — the main enzyme of metabolism of thyroid hormones in the liver of rats upon the retained function of the thyroid gland.

The dogs were considered as the most suitable species of animals for extrapolation of data, considering changes in the liver, eyes, nervous system, changes in the level of T4, glucose, albumins. The reduction of the levels of T4 and T3 in the blood was considered as the indicator of sensitivity of flufenacet effect. Physiological sensitivity of dogs to changes of these hormones and their homeostasis is closer to human homeostasis than that in rats [11].

However, considering the principles of hygienic rating set out in Ukraine, bases of which are formed by the principles of aggravation and sub-threshold (lack) of effects, upon justification of ADI, sub-threshold dose established at the most sensitive species of animals — rats — was taken into account.

As a result of the conducted analysis, ADI for flufenacet was justified at the level of 0.004 mg/kg, considering:

— minimal NO(A)EL of 1,2 mg/kg established in the chronic experiment in rats and NO(A)EL 1,14 mg/kg, established in the experiment in dogs;
— safety factor 300 (considering the presence of cataractogenicity and neurotoxic action in 3 species of animals; more pronounced sensitivity of young animals to the substance action; deviation detected in the experiment on the investigation of embryoneurotoxicity).

Justified level of ADI provides safety factor in respect to the minimal concentration in the experiment investigating embryotoxicity and teratogenicity — 1250–6250, reproductive toxicity — 9350, carcinogenicity — 9750–15550.

It should be noted that in 2003 the level of acceptable daily intake (ADI) for flufenacet at the level of 0,005 mg/kg was set out in the European Union [10], considering the level of the least threshold dose, established in the chronic experiment in rats (1,2 mg/kg), with the safety factor of 250.

In Canada [11], ADIwas justified at the level of 0,0038 mg/kg, considering minimal threshold dose of 40 ppm (1.14 mg/kg) established in the experiment in dogs and safety factor of 300 (due to the lack of sub-threshold

dose in the main experiment). This value provides safety factor (MOE) 1,850 in respect to the sub-threshold doses in adults upon the study of reproductibility and neurotoxicity.

Conclusion. It was established that according to DSanPin 8.8.1.002-98, flufenacet, according to the parameters of acute oral toxicity and percutaneous toxicity, belongs to class 4 (low hazardous), according to acute inhalation toxicity — to class 2 (hazardous), according to skin irritation — to class 3 (has a mild irritant effect), according to mucosa irritation — to class 4 (has no irritant effect), and according to allergenic action — to class 4 (is not an allergen).

1. Liver was considered as the main target organ of the effect of flufenacet (hepatocellulary hypertrophy), and the secondary one — the thyroid gland (reduced concentration of thyroxine, triiodothyronine).

2. It was established that flufenacet has no effect on the function of the pituitary gland and the thyroid gland, and it induces liver metabolism of thyroid hormones by phenobarbital type leading to the reduction of blood T4 level.

3. ADI of flufenacet for human was justified at the level of 0,004 mg/kg (minimal NO(A)EL 1,2 mg/kg, established in the chronic experiment in rats and NO(A)EL 1,14 mg/kg, established in the experiment in dogs, safety factor — 300).

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