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HYGIENIC ASSESSMENT OF RISK CAUSED BY APPLICATION OF GRAMINIS KE AND RINKOR VG HERBICIDES

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Our research goal was to perform hygienic assessment of risks caused by Graminis KE and Rinkor VG herbicides for people working with them. We applied sanitary-hygienic and toxicological research techniques in our work in full conformity with valid technical regulatory documents and guidelines. We set the following research tasks: to analyze literature and information sources; to perform primary toxicological assessment of preparatory herbicides and study their acute toxicity
together with sensitizing effects at intragastric introduction, cutaneous application, and inhalation exposure on laboratory animals; to examine herbicides cumulative effects and calculation their cumulation coefficient; to examine working conditions during a natural experiment when Graminis KE and Rinkor VG herbicides were applied and calculate risks for workers; to work out scientifically grounded recommendations on their safety application in agriculture.

The examined herbicides, Graminis KE and Rinkor VG, are classified as substances with the 3rd hazard degree as per their toxicometric parameters (moderately hazardous substances). Calculated risks of complex (inhalant and dermal) exposure to Gramins KE and Rinkor VG herbicides for workers (operators who refills them and those who spray plants with them) when they are applied in agriculture don't exceed acceptable levels (are less than 1). Our work results allow to enrich a set of plant protectors which are applied in the country and to use such preparations in agriculture which are the least harmful for health and the environment. Application of Graminis KE and Rinkor VG herbicides will help to increase crops productivity.

Key words: *hygienic risk assessment, herbicides, primary toxicological assessment, acute toxicity, sensitizing effects, cumulative properties, cumulation coefficient, agriculture.*

History of agriculture gives us an deniable proof that such extermination activities as hand weeding, cultivation, and harrowing, had always been playing the leading role in fighting against weeds. But then herbicides were invented and it led to a real revolution in agriculture and old techniques to a certain extent were replaced with new ones. Agricultural workers all over the world have been applying herbicides for more than 60 years completely relying on them; we can say that herbicides are now among most widely spread agricultural chemicals [1, 2, 4, 9].

Contemporary crop production involves certain tasks; for example, it is vital to achieve maximum possible productivity of crops but ensuring here that agricultural products don't contain any substances which can be toxic for people and animals, for example, pesticides [5].

Crops protection means are mostly chemicals which are created and applied for fighting weeds and pests. Pesticides appli-

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cation allows to produce stable crops and to limit spread of infections which are transferred by carrying agents, for example, malaria and epidemic typhus. However, unconsidered application of pesticides has also negative consequences and results in greater resistance to them among organisms, especially insects; it can kill natural enemies of pests and other beneficial animals. Pesticides contaminate the environment and are a threat for human health. Herbicides take the first place among crop protectors as per applied quantities [7].

Herbicides is a commonly and worldwide used name for chemical crop protectors; it consists of two basic words: herb for plant and cide for eliminate, that is a plant-eliminating substance [3].

About 4.5 million tons of various herbicides are produced annually all over the world; they are intensively put into soils, especially in the regions with highly developed agriculture, and, consequently, exert negative influence on the environment in them. Herbicides are accumulated in soils. They can be washed out of them, penetrate natural water reservoirs and then they can be introduced into human and animals bodies. Physical and chemical absorption, biological and enzymatic destruction play their own role in soils self-purification from poisons; however, some toxins with systemic effects can penetrate into crops and create threats for food and fodders quality [13–15].

Chemical components are known to inhibit live activity of soils biological component, namely bacteria, fungi, ray fungi, algae, rhizopod, flagellates, etc., living in them and participating in humus creation. As these soils inhabitants perish, it makes soils less nutritious. And effects exerted by chemicals can be rather long-term [16].

Herbicides, in comparison with other crop protectors, are more phyto-toxic which is characteristic for substances with weak selective effects.

Sometimes substances with wide range of effects eliminate not only weeds but also useful cultures which are related to a target object (the same family etc.). For example, when beetroot crops are treated, certain substances kill not only lamb's-quarters, but also beetroot itself; when herbicides exert their impacts on monocotyledonous weeds, they can also harm cultivated cereal crops. To preserve a cultivated culture, agricultural workers usually choose either substances which beneficial crops are resistant to, or those which have maximum selective effects [11, 12, 17].

Growing concern related to pesticides abuse led to creation of basic rules for their application accepted in many countries all over the world. These rules comprise all the aspects of their distribution: transportation, storage, packing utilization, and maximum permissible residual quantities.

There is a law issued in the EU which forbids application of highly toxic pesticides, including carcinogenic and mutagenic substances which cause reproductive function disorders and endocrine systems disorders, and which are persistent and capable of biological accumulation.

Experts allow for high threats of herbicides contamination, both during their application and manufacturing and transportation and therefore recommend to apply maximum safe or less toxic pesticides, or try to resort to non-chemical alternative pests-fighting techniques, such as cultivating, biological preparations (for example, pheromones and microbe pesticides), genetic engineering, genetic modifications, wastes composting, etc. These techniques are becoming more and more popular and are often less dangerous than conventional chemical pesticides.

Therefore, nowadays it is advisable to offer a wide choice of promising crop protectors to agricultural workers; these crop protectors will allow to minimize negative influence on population health and the environment and to lower economic damage related to it; they will also give grounds for manufacture of ecologically safe food products.

In order to prevent negative consequences of herbicides application, to give scientific foundations for risks caused by it in real life conditions, and to develop measures aimed at herbicides safe distribution, it is necessary to perform toxicological and sanitary-chemical examinations of new preparations as it will help to minimize negative influence exerted by them on population health and the environment and to lower economic damage related to it, and to achieve their maximum efficiency in fighting against weeds growing on fields with agricultural crops.

Our research goal was to perform hygienic assessment of risks caused by two herbicides, Graminis, CE (concentrated emulsion) and Rinkor, WG (water-soluble granules) for workers involved in their application.

We solved the following tasks in the course of our research: to analyze literature and information sources; to make primary toxicological assessment of herbicides preparatory forms with experiments on laboratory animals aimed at studying acute toxicity at intragastric introduction, cutaneous application, and inhalation exposure, and sensitizing effects caused by it; to examine herbicides cumulative properties and calculate cumulation coefficient; to study working conditions during a natural experiment at Graminis CE and Rinkor WG herbicides application and calculate risks for workers; to develop scientifically grounded recommendations on their safe application in agriculture.

Data and methods. We applied sanitary-hygienic and toxicological research techniques (acute toxicity at intragastric introduction, cutaneous application, and inhalation exposure; sensitizing effects; cumulative properties) in conformity with valid technical regulatory legal acts and guidelines [6, 8]. Results were statistically processed with the use of MS Excel XP and Statistica 6.0 software.

Results and discussion. We studied acute toxicity at intragastric introduction in the experiment in accordance with the Instruction No. 1.1.11-12-35-2004[1] (Chapter 4) with the following doses: 3980, 5010, 6340 and 7940 mg/kg. Each dose in acute experiments was tested on 6 animals (males) at intragastric introduction via a needle probe with the following observation during 14 days. Experiments were performed on sexually mature white rats with body weight being equal to 190-220 grams. We considered intoxication symptoms, animals' behavior and death. Clinical picture of acute intoxication during the first few hours after introduction included heavy breathing, inhibited movements, and insignificant tremor; the symptoms became worse during the first day and later were accompanied with shaky gait, palpebral fissure narrowing, convulsive breathing, refusal to eat, and lateral position. Animals died from respiratory center stop on 1-2 day after the introduction. Rinkor, WG DL50 (lethal dose) calculated as per V.B.

[1] Instruction No. 1.1.11-12-35-2004. Requirements for experimental research aimed at primary toxicological assessment and hygienic regulation of substances. Approved by the Public Healthcare Ministry in Republic of Belarus dated December 14, 2004. 42 p. Available at: http://www.vniiki.ru/document/4689864.aspx (16.05.2017).

Prosorovskiy technique [10] amounts to 5210 (4400÷6100) mg/kg; Graminis, CE DL50, 5620 (4300÷7400) mg/kg. So, preparatory forms of these two herbicides should be considered low-hazard chemicals as per their acute intragastric toxicity (IV danger category as per State Standard 12.1.007-76[2]).

Examination of acute toxicity at cutaneous application. The experiment was performed in conformity Application Instruction No. 048-1215[3] on white sexually mature rates (males and females, n=6), with body weight equal to 190–210 grams. Herbicides preparatory forms were once applied on skin areas, 4x4 cm each, in the middle one-third of a dorsal body surface where fur was cut off; the areas were then covered with gauge bandage and fixed by adhesive plaster. Animals were put into individual cages. Exposure on skin lasted on 24 hours; after that skin was washed with distilled water. We applied the following dose of Rinkor, WG: 1500 (experiment 1), 2000 (experiment 2), 2500 (experiment 3) mg/kg; Graminis, CE: 2000 (experiment 1), 2500 (experiment 2), 5000 (experiment 3) mg/kg. There were 6 animals in each group. We observed them for 14 days and considered intoxication symptoms nature, body weight dynamics, and animals behavior. Clinical picture of acute intoxication during the first hours after application included insignificant decrease in locomotor activity; animals behavior didn't have any discrepancies from the control group on the next day and during the whole observation period. No animals died during the whole 14-day observation period. Therefore, herbicides preparatory forms should be considered low-hazard chemicals as per their acute cutaneous toxicity parameters (IV danger category as per State Standards 12.1.007-76[2]).

Examination of acute toxicity under inhalation exposure. This experiment was performed on sexually mature white rats with body weight equal to 190-200 grams in conformity with the Application Instruction No.047-1215[4]. Preparatory forms of herbicides were applied in concentrations equal to 0.5; 1.0; 2.5, and 5 mg/l. Inhalation lasted for 4 hours including balancing period. Each dose in acute experiments was tested on 6 animals, 3 males and 3 females, which were then under observation for 14 days. We considered intoxication symptoms, body weight dynamics and animals' behavior. Animals which received herbicides in maximum concentration under inhalation exposure had lower motor activity, their fur was disheveled, and they also had excretion from their noses which stopped during the first day after the exposure was over. All the tested doses didn't cause any deaths. CL50 (a substance concentration which causes deaths of 50% animals under two-, four-hour inhalation exposure) amounts to more than 5.000 mg/l (5000 mg/m^3). Therefore preparatory forms of herbicides can be considered moderately dangerous chemicals as per their acute inhalation toxicity (III danger

[2] State Standard 12.1.007-76. Labor Safety Standards System (LSSS). Hazard substances. Classification and general safety requirements (with Alterations N 1, 2): Interstate standard. Available at: http://docs.cntd.ru/document/5200233 (18.05.2017)

[3] Application Instruction No. 048-1215. Determination of chemicals acute toxicity (chemicals and their mixtures) at cutaneous application. Approved by Deputy to Public Healthcare Minister – Chief Sanitary Inspector of Republic of Belarus on August 30, 2016. Available at: http://rspch.by/Docs/048-1215.pdf (20.05.2017).

[4] Application Instruction No. 047-1215.Determination of chemicals acute toxicity (chemicals and their mixtures) under inhalation exposure. Approved by Deputy to Public Healthcare Minister – Chief Sanitary Inspector of Republic of Belarus on August 30, 2016. Available at: http://rspch.by/Docs/047-1215.pdf (20.05.2017).

category as per State Standard 12.1.007-76[2]).

Sensitizing effects. The experiment was performed in conformity with the Instruction 1.1.11-12-35-20041 (chapter 8). Intracutaneous introduction of Rinkor WG preparatory form in a challenging dose equal to 100 μl into a hindleg pad (below the aponeurosis) of white mice didn't lead to edematic-proliferative reaction evolvement. We measured a pad width in mm before and 24 hours after percutaneous testing with the use of an engineering micrometer. A reaction appearance was assessed both as per absolute (mm) and relative (scores) parameters. Average parameters of a mouse pad swelling test (allergic test) in animals from the focus group didn't have any discrepancies from those in the relevant control group and didn't exceed 0.1 mm (0 scores) (< 0.05). Intracutaneous introduction of Graminis CE herbicide preparatory form in a challenging dose caused edematic-proliferative reaction evolvement. Average parameters of a mouse pad swelling tests differed from those in the relevant control group and amounted to 0.143-0.198 mm (1 score) (<0.05). The experiment enabled determining that the examined Rinkor WG herbicides preparatory form didn't cause any induration or inflammation in tissues while Graminis CE herbicide preparatory form did as much as a result of tissues infiltration caused by interaction between an antigen (allergen) with macrophages and Thl-lymphocytes which stimulated cellular immunity. So, Rinkor WG herbicide preparatory form can be considered a substance without any sensitizing effects (4 category, no sensitizing effects); Graminis CE is a substance causing weak sensitizing effects (3 category (3B subcategory) weak allergen).

Irritating effects on eyes mucous tunics under a single exposure. The experiment was performed in conformity with the Instruction 1.1.11-12-35-2004[1](chapter 5) and the Instruction No. 045-1215[5].

Preparatory forms of herbicides in doses equal to 50-100 μl were introduced into a low conjunctival fornix of rabbits' right eyes; 24 hours later the eyes were washed with distilled water; left eyes were taken as control ones and 1-2 drops of distilled water were introduced into them. The examined substances caused lacrimation, serous excretions, and vessels injection after instillation; all these effects stopped on the 1-2 day after washing with water when the exposure ended. Mucous tunics apparently recovered fully during 1-2 days after instillation. So, in case of a single exposure on mucous tunics Rinkor WG herbicide preparatory form exerts weak irritating effects on mucous tunics with average total scoring showing irritating effects evidence being equal to 1.0 (3 category (3B subcategory) weak irritating effects); Graminis CE exerts moderate irritating effects on mucous tunics with average total scoring showing irritating effects evidence being equal to 3.0 (3 category (3A subcategory) moderate irritating effects).

Examination of local irritating properties on skin under a single exposure. The experiment was performed in conformity with the Instruction 1.1.11-12-35-2004[1] (chapter 6) and the Instruction No. 049-1215[6]. Preparatory forms of herbicides were put on bare skin on backs of non-linear white rats, spots on animals backs were located on the right side, their square was 4×4 cm (left side was taken as

[5] Application Instruction No. 045-1215. Determination of chemicals acute irritating effects (chemicals and their mixtures) on eyes mucous tunics. Approved by Deputy to Public Healthcare Minister – Chief Sanitary Inspector of Republic of Belarus on August 30, 2016. Available at: http://rspch.by/Docs/045-1215.pdf (20.05. 2017).

control); doses were equal to 20 mg/cm2 and were applied only once with application duration equal to 4 hours. We considered skin state and how apparent skin reactions were. Preparatory forms of herbicides under a single exposure on bare spots located on rats' backs didn't exert any irritating effects scoring 0 (4 category) (absence of irritating effects).

Cumulative properties assessment. The experiment was performed in conformity with the Instruction No. 052-1215[7] on white male rats with body weight equal to 170–200 grams, aged 8–12 weeks, which we bred ourselves. The total number of experimental animals was 14 (7 male rats in the focus group and 7 in the control one). To assess cumulative properties of herbicides preparatory form, we chose a dose equal to ≈1/10 from the maximum dose introduced in an acute experiment. Rinkor WG herbicide dose was equal to 500 mg/kg of body weight; Graminis CE, 550 mg/kg of body weight. We didn't detect any animals' deaths during the whole experiment. So, the examined preparatory forms don't have any cumulative properties with lethal effects (cumulation coefficient > 5).

All the detected statistically significant changes in some morphofunctional parameters chosen for assessing toxicological effects exerted by the examined substances after 90-days intragastric re-introduction are most likely to be compensatory-adaptive.

We performed microscopic pathomorphologic examination of internal organs and didn't detect any visible changes. We determined that liver and kidneys were most typical target organs for sulfonylurea (Rinkor WG and Graminis CE herbicides).

We performed hygienic assessment of working conditions at workplaces where herbicides were applied on a test field during a single spraying via a tractor rod sprayer, potatoes tops being 5-25 cm high, discharge rate being 50 g/hectare, and working fluid discharge being 250 l/hectare (Rinkor WG), and 50 g/hectare, 200 l/hectare, correspondingly for Graminis CE. Work lasted for 40 minutes; working shift duration was 6 hours; daily rate of a treated square was 25 hectares.

The experiment allowed to detect that substances contaminated certain spots on workers bodies, both protected with individual protection means (neck and breast) and unprotected (face). In relation to that average dermal load (concentration) for the rest chosen spots was calculated allowing for ½ detection limit for samples having "not detected" value and amounted to 0.00125 mg/m2 for herbicides contaminating bodies of a refilling operator and a sprayer operator. Workers didn't feel themselves bad; they didn't have any skin irritations or eyes mucous tunics irritation; they didn't complain on any health deterioration after work.

We performed hygienic assessment of risks for people contacting the examined substances during potatoes corps treatment with discharge rate being equal to 50 g/hectare in conformity with the Methodical guidelines No. 2001/73 [7]. Risks of

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[6] Application Instruction No. 049-1215. Determination of chemicals acute irritating effects on skin (chemicals and their mixtures). Approved by Deputy to Public Healthcare Minister – Chief Sanitary Inspector of Republic of Belarus on August 30, 2016. Available at: http://rspch.by/Docs/2_049.pdf (20.05. 2017)

[7] Application Instruction No. 052-1215. Determination of chemicals toxicity (chemicals and their mixtures) and repeat and chronic intragastric introduction. Approved by Deputy to Public Healthcare Minister – Chief Sanitary Inspector of Republic of Belarus on August 30, 2016. Available at: http://rspch.by/Docs/052-1215.pdf (20.05.2017)

adverse effects caused by the substances exposure on skin were determined via comparison between actual skin exposure (Eac, mg/cm2), calculated on the basis of the obtained data and tentatively permissible level of skin contamination (TPLs.c., mg/cm2) (Table).

Total risks for a refilling operator and a sprayer operator under cutaneous and inhalation exposure to herbicides during potatoes crops treatment

Calculated coefficients	The substance			
	rimsulfuron (Rinkor WG)		chisalofop-p-ethyl (Graminis CE)	
	A sprayer operator	Refilling operator	A sprayer operator	Refilling operator
Safety coefficient under cutaneous pesticide introduction dermal exposure risk), $SC_{d.}$	0,018000	0,028000	0,029	0,029
Safety coefficient under inhalation introduction (inhalation exposure risk), $SC_{inh.}$	0,003333	0,000333	0,075	0,075
Total risk, $SC_{total.}$	0,021333	0,028333	0,104	0,104
Standard total risk value, $SC_{total.}$	< 1	< 1	< 1	< 1

Notes: $E_{ac.}$ is actual skin exposure, mg/cm^2; $MPC/TSEL_{w.a.a.}$ is maximum permissible concentration/tentatively safe exposure level in work area air

Conclusions:

1. Preparatory forms of herbicides should be considered low-hazard chemicals as per their acute intragastric toxicity (IV danger category as per State Standard 12.1.007-76).

2. Preparatory forms of herbicides should be considered low-hazard chemicals as per their acute cutaneous toxicity (IV danger category as per State Standard 12.1.007-76).

3. Preparatory forms of herbicides should be considered moderately hazardous chemicals as per their acute inhalation toxicity (III danger category as per State Standard 12.1.007-76).

4. Pinkor WG herbicide preparatory form can be considered a substance without any sensitizing effects (4 category, absence of sensitizing effects); Graminis CE, a substance with weak sensitizing effects (3 category (3B subcategory), weak allergen).

5. Herbicide Rinkor WG preparatory form under a single exposure on mucous tunics exerts weak irritating effects on them with total scoring showing irritating effect evidence being equal to 1.0 (3 category (3B subcategory), weak irritating effects); Graminis CE exerts moderate irritating effects on mucous tunics with total scoring showing irritating effect evidence being equal to 3.0 (3 category (3A subcategory), moderate irritating effects).

6. Herbicides preparatory forms don't exert any irritating effects on skin as there were no such effects on bare skin spots located on white rats' backs; scoring is 0 (4 category), absence of irritating effects).

7. The examined preparatory forms don't have any cumulative properties with lethal effects (cumulation coefficient is > 5).

8. We didn't detect any changes in internal organs via microscopic pathomorphologic examination.

9. Calculated risks of complex (inhalation and dermal) effects exerted by Graminis CE and Rinkor WG herbicides on workers (a refilling operator and a sprayer operator) during their application in agriculture don't exceed permissible levels (are less than 1).

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