EXPERIMENTAL MODEL AND MEASUREMENT STUDIES OF RISK ASSESSMENT IN HYGIENE AND EPIDEMIOLOGY

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PECULIARITIES OF EPICUTANEOUS ACTION OF HEXYL ETHER OF 5-AMINOLEVULINIC ACID

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We studied the subacute toxicity of hexyl ether of 5-aminolevulinic acid at the epicutaneous action in the experiment with white rats. It was established that repeated application of hexyl ether of 5-aminolevulinic acid cause the weak reaction of the white rats skin in the form of edema. The transdermal hexyl ether of 5-aminolevulinic acid intake route is characterized by the increase in the alanine aminotransferase activity, the level of urea in the blood serum and shift to the acid side of pH in the urine of experimental animals. The exposure dose of 341 mg/kg is the dose acting with minimum deviations of values and is accepted as threshold. The exposure dose of 75 mg/kg does not cause the changes in the condition of laboratory animals and is the maximum inactive dose.

Key words: toxicity, hexyl ether of 5-aminolevulinic acid, epicutaneous impact.

One of the methods of modern agronomic technologies in the plant cultivation is the use of the plants growth regulators to increase the yield of agricultural crops. During the development and manufacture of such formulations it is necessary to pay special attention to their environmental safety. Principally new approach to the creation of environmentally-friendly plant growth regulators is the use of the natural metabolites of plants for this purpose, in particular 5-aminolevulinic acid (ALA). ALA is the primary intermediate of all cyclic (chlorophylls, hemes, corrinoids) and linear (bilins, phycobilins) tetrapyrroles which play an exclusive role in the metabolism of plant, animal and bacterial bodies [5]. At the end of 80-is in the last century for the first time is was found that ALA has the growth regulator properties [4]. The ALA efficiency is significantly increased by the lipofilization of molecule during its use in the form of ethers with higher alcohols that is implemented by the scientists of the Bioorganic Chemistry Institute of the National Academy of Sciences of Belarus as a result of development of laboratory technique for the obtainment of hexyl ether of ALA (HE-ALA). The field experiments established that the use of HE-ALA has the positive impact on the growth and development of plants under a number of indicators, herewith the effect was achieved at the concentrations 5 times (amd more) lower than during the ALA treatment [2].

For safe use of HE-ALA in the agro-industrial complex it is necessary to have its complete toxicological and hygienic assessment that will allow for minimizing the risks for persons working in the field of agriculture and production. Herewith, the special role belongs to toxicological experiments on laboratory animals which result in determining

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the leading feature of harmful effect and establishing the threshold doeses or concentraions.

The purpose of this work is to study the peculiarities of the HE-ALA epicutaneous impact since its contact with skin can have potential hazard. During the experiment we assessed the manifestation of local irritating and skin resorptive properties at the repeated applications for white rats.

Materials and methods. The study is performed in the repated experiments on 28 males of random species of white rates with weight of 180-215 g during 1 month. The healthy animals with clean body hair coat after group adaptation were selected for the experiment. The day before the beginning of the experiment the hair on the back of rats was cut. The animals were weighed and divided into four groups, each group consisted of 7 animals: I – control and II, III, IV – experimental exposed to 50%, 25% and 5% (m/V) HE-ALA solutions, respectively. The solvent – distilled water – was applied to the control group of rats. The study of local irritating and skin resorptive properties was performed through the application of 320 µl HE-ALA on the clipped areas of skin 4´ 4 cm in the aqueous solution (0.02 ml/cm^2). The animals were subjected to exposure for 4 hours 5 days a week. At the end of daily exposure the substance was washed with warm water. Access to food and water was free and the lighting was natural. During the experiment we assessed the signs of systemic toxicity and local changes. The intensity of erythema was assessed visually in points: absence – 0, low – 1, moderate intensity – 2, intensive – 3, extremely intensive – 4. The cutaneous edema volume was determined by measurement of skin fold with micrometer (in mm), its intensity compared to background value was assessed under the scale from 0 to 4 points [3]. After the completion of experiment the rats were weighed and after the immediate decapitation the weight coefficinent of organs were determined and the changes of internal organs were macroscopically assessed. The obtained blood was tested using the hematological analyzer Mythic18 (Switzerland), the biochemical indicators of the blood serum and urine were determined on the automatic analyzer Accent200 (Poland). AST/ALT ratio as well as creatinine and urine clearances were calculated using the standard methods [1].

The analysis of data for the compliance with normal distribution law is performed using Kolmogorov-Smirnov criterion. During the assessment of differences between groups we used the parametrical *t*-criterion of Student taking into account Bonferroni correction. The quantitative parameters are presented in the form of mean value (*M*) and 95% confidence interval (95% CI). $p \leq 0.05$ was accepted as the critical level of significance during the check of statistical hypothesis.

Results and their discussion. The mean value of exposure dose in terms of the weight of animal on the 30th day of experiment for groups was as follows: II – 864 (821-906) mg/kg, III – 341 (318-363) mg/kg, IV – 75 (70-81) mg/kg HE-ALA.

When studying the local irritating action of HE-ALA during 30 days of experiment no signs of hyperemia and visible changes in the skin of experimental animals were observed. The areas of skin at the places of applications were similar to control, and did not have the indurations, exfoliation or foreign formations. At the end of experiment the skin fold thickness was determined for all the animals (table 1).

Table 1

Results of the skin fold thickness measurement in white rats exposed to epicutaneous impact of HE-ALA during 30 days, mm, *M* (±95% CI)

Group	Skin fold thickness value, mm		Increase of the skin fold thickness	
	before the beginning of experiment	on the 30th day of experiment	during experiment, mm	compared to control, %
I (control)	1,4 (1,3–1,6)	1,6 (1,5–1,8)	0,19 (–0,03–0,4)	100
II	1,3 (1,2–1,5)	1,7 (1,6–1,8)	0,37 (0,17–0,57), *p*=0,68	195
III	1,6 (1,4–1,8)	1,8 (1,7–2,0)	0,24 (0,02–0,46), *p*=1,0	126
IV	1,6 (1,5–1,7)	1,8 (1,6–1,9)	0,16 (0,02–0,3), *p*=1,0	84

Table 2

Dynamics of change in the live weight of white rats exposed to epicutaneous impact of HE-ALA during 30 days, kg^{-1}, *M* (±95% CI)

Group	Animal body weight, kg^{-1}		Body weight increase	
	before the beginning of experiment	on the 30th day of experiment	during experiment, kg^{-1}	compared to control, %
I (control)	186,4 (182,0–190,8)	210,0 (195,9–224,1)	23,6 (12,7–34,5)	100
II	181,4 (177,9–184,9)	185,7 (176,7–194,7)	4,3 (–6,2–14,8), *p*=0,11	18
III	200,7 (192,1–209,3)	235,7 (220,8–250,7)	35,0 (23,4–46,6), *p*=0,86	148
IV	197,9 (192,6–203,1)	214,3 (197,5–231,1)	16,4 (–1,4–34,3), *p*=1,0	69

According to the data of table 1, HE-ALA did not cause the statistically significant deviations in the skin fold thickness between the control and experimental groups. In addition, taking into account the dose-dependent effect, it is possible to give 1 point (low reaction) to group II under the intensity of edema the difference of which compared to control is 0.18 mm or 195%. Visually the signs of erythema were absent in all the groups (0 points). Therefore, the total quantitative assessment of the erythema and edema induction degree for I group and III, IV groups obtained 25% and 5% HE-ALA is 0 points, and for II group of rats obtained 50% HE-ALA – 1 point.

When studying the skin resorptive properties of HE-ALA it was established that 30-fold epicutaneous HE-ALA applications does not result in the death of experimental animals. General condition of animals was satisfactory and no deviations in the behavioral activity were recorded. No visible changes of internal organs were found during the postmortem examination.

The increase of body weight was observed in all the experimental groups at the end of experiment. The lowest increase is established in II group obtained 50% HE-ALA solution. It should be noted that the separate animals of II and IV group had negative body weight increase dynamics compared to the intitial data (table 2).

The differences in the increase of body weight on the 30th day of experiment in the control and experimental groups are not statistically significant, and the dose-dependent effect is absent.

The biochemical indicators of blood serum and urine as well as the morphological blood composition to the fullest extent reflect the metabolic activity condition. According to table 3, the morphofunctional blood indicators for the animals of groups obtained the cutaneous HE-ALA during 30 days did not differ from control.

Table 3

Morphofunctional blood indicators for white rats exposed to epicutaneous impact of HE-ALA during 30 days, *M* (±95% CI)

Indicator	Group			
	I	II	III	IV
Leukocytes, 10^9 kl/l	14,6 (9,3–19,3)	21,2 (12,2–30,2), *p*=0,46	15,7 (9,9–21,4), *p*=1,0	12,4 (6,0–18,9), *p*=1,0
Lymphocytes, 10^9 kl/l	9,6 (6,1–13,2)	15,5 (6,8–24,3), *p*=0,33	9,0 (6,2–11,8), *p*=1,0	7,6 (4,6–10,5), *p*=1,0
Monocytes, 10^9 kl/l	0,57 (0,44–0,69)	0,7 (0,52–0,88), *p*=0,76	0,6 (0,4–0,7), *p*=1,0	0,5 (0,3–0,8), *p*=1,0
Granulocytes, 10^9 kl/l	4,4 (3,3–5,6)	4,9 (4,4–5,5), *p*=1,0	6,1 (0,8–11,4), *p*=1,0	4,4 (1,0–7,7), *p*=1,0
Erythrocytes, 10^{12} kl/l	7,9 (7,5–8,3)	8,3 (7,9–8,7), *p*=0,89	7,6 (7,1–8,0), *p*=0,88	7,8 (7,0–8,6), *p*=1,0
Hemoglobin concentration, g/l	143,3 (136,0–150,6)	153,2 (143,2–163,2), *p*=0,4	135,3 (125,0–145,6), *p*=0,77	144,5 (131,9–157,1), *p*=1,0
Hematocrit, l/l	0,38 (0,37–0,39)	0,4 (0,38–0,42), *p*=0,57	0,36 (0,33–0,39), *p*=1,0	0,38 (0,34–0,42), *p*=1,0
Average erythrocyte volume, fl	47,9 (46,2–49,6)	48,5 (46,9–50,1), *p*=1,0	47,7 (46,2–49,2), *p*=1,0	48,7 (46,8–50,6), *p*=1,0
Average content of hemoglobin in erythrocyte, pg	18,1 (17,0–19,2)	18,5 (17,6–19,3), *p*=1,0	17,9 (17,4–18,4), *p*=1,0	18,6 (18,2–19,0), *p*=1,0
Average concentration of hemoglobin in erythrocyte, g/l	377,8 (367,4–388,3)	381,0 (376,3–385,7), *p*=1,0	375,0 (370,9–379,0), *p*=1,0	380,5 (366,7–394,3), *p*=1,0
Thrombocytes, 10^9 kl/l	677,5 (462,8–892,2)	519,2 (358,6–679,7), *p*=0,57	540,8 (451,8–629,8), *p*=0,87	566,5 (318,1–814,9), *p*=1,0
Average thrombocytes volume, fl	6,3 (5,8–6,8)	6,3 (5,9–6,8), *p*=1,0	6,3 (6,1–6,5), *p*=1,0	6,2 (5,5–6,9), *p*=1,0

Table 4

Biochemical blood serum indicators for white rats exposed to epicutaneous impact of HE-ALA during 30 days, *M* (±95% CI)

Indicator	Comparison groups			
	I	II	III	IV
Total protein, g/l	78,8 (67,1–90,5)	63,8 (45,5–82,0), *p*=0,56	63,6 (49,8–77,5), *p*=0,54	90,0 (62,7–117,3), *p*=1,0
Albumin, g/l	48,5 (36,5–60,5)	52,7 (39,6–65,7), *p*=1,0	45,1 (31,9–58,3), *p*= 1,0	37,5 (13,0–61,9), *p*=1,0
Urea, mMol/l	6,8 (6,0–7,6)	8,3 (7,1–9,5) *, *p*=0,032	6,5 (5,8–7,2), *p*=1,0	7,6 (7,0–8,3), *p*=0,9
ALT, mccat/l	1,62 (1,43–1,8)	1,97 (1,73–2,22)*, *p*=0,045	2,1 (1,91–2,22)*, *p*=0,002	1,72 (1,55–2,23), *p*=0,99
AST, mccat/l	5,16 (4,39–5,92)	6,31 (5,06–7,55), *p*=0,28	5,88 (5,09–6,67), *p*=1,0	6,92 (5,33–8,52), *p*=0,053
AST/ALT ratio, conventional units	3,24 (2,63–3,85)	3,20 (2,70–3,70), *p*=1,0	2,79 (2,52–3,06), *p*=0,34	3,11 (2,60–3,63), *p*=1,0
Cholesterol, mMol/l	1,9 (1,4–2,4)	1,9 (1,7–2,2), *p*=1,0	1,2 (1,1–1,4) *, *p*=0,01	1,8 (1,3–2,4), *p*=1,0
Creatinine, mcMol/l	43,0 (40,4–45,6)	40,31 (37,9–42,7), *p*=0,18	41,1 (38,2–44,0), *p*=0,81	40,2 (38,1–41,9), *p*=0,14
Uric acid, mcMol/l	75,7 (58,9–92,4)	101,5 (80,7–122,3), *p*=0,19	87,5 (69,6–105,4), *p*=1,0	86,0 (45,6–126,4), *p*=1,0
α-amilase, mccat/l	16,0 (12,65–19,34)	13,97 (10,12–17,82), *p*=1,0	14,35 (9,89–18,81), *p*=1,0	14,55 (11,34–17,76), *p*=1,0
Alkaline phosphatase, mccat/l	1,85 (1,64–2,06)	1,81 (1,76–1,85), *p*=1,0	1,95 (1,78–2,11), *p*=1,0	1,83 (1,64–2,03), *p*=1,0
Glucose, mMol/l	6,6 (5,5–7,7)	7,3 (6,5–8,1), *p*=1,0	7,6 (7,1–8,2), *p*=0,42	7,3 (5,0–9,6), *p*=1,0
Calcium, mMol/l	5,0 (4,5–5,5)	5,1 (4,7–5,5), *p*=1,0	4,6 (4,2–4,9), *p*=1,0	6,7 (1,8–11,7), *p*=0,35
Magnesium, mMol/l	0,94 (0,82–1,1)	0,98 (0,9–1,1), *p*=1,0	0,89 (0,79–1,0), *p*=1,0	0,99 (0,81–1,2), *p*=1,0
LDH, mccat/l	82,1 (51,9–112,3)	78,7 (50,7–106,8), *p*=1,0	58,9 (46,0–71,7), *p*=0,56	62,0 (35,5–88,4), *p*=1,0
GGT, mccat/l	0,19 (0,16–0,21)	0,19 (0,18–0,2), *p*=1,0	0,19 (0,17–0,22), *p*=1,0	0,19 (0,17–0,2), *p*=1,0

Note: * – the differences are statistically credible, $p \leq 0.05$.

Table 5

Urinary system indicators for white rats exposed to epicutaneous impact of HE-ALA during 30 days, *M* (±95% CI)

Indicator	Groups			
	I	II	III	IV
Total protein, g/l	7,5 (3,6–11,4)	10,3 (5,4–15,1), *p*=1,0	8,2 (5,2–11,1), *p*=1,0	8,1 (0,1–16,2), *p*=1,0
Urea, mMol/l	96,7 (32,7–160,6)	150,3 (90,2–210,4), *p*=0,94	134,7 (58,3–211,8), *p*=1,0	126,8 (35,9–217,6), *p*=1,0
Clearance of urea, ml/min	0,12 (0,07–0,17)	0,11 (0,06–0,17), *p*=0,94	0,10 (0,06–0,14), *p*=1,0	0,12 (0,09–0,15), *p*=1,0
Creatinine, mMol/l	4,8 (2,4–7,1)	3,8 (3,1–4,5), *p*=1,0	3,1 (2,5–3,6), *p*=0,3	7,1 (4,7–9,6), *p*=0,33
Clearance of creatinine, ml/min	0,68 (0,44–0,98)	0,67 (0,51–0,83), *p*=1,0	0,81 (0,48–1,15), *p*=1,0	0,75 (0,57–0,92), *p*=1,0
Uric acid, mcMol/l	456,5 (184,6–728,4)	826,3 (634,7–1017,9), *p*=0,22	908,5 (514,2–1302,9), *p*=0,08	762,0 (315,5–1208,6), *p*=0,67
Glucose, mMol/l	0,95 (0,63–1,26)	1,1 (0,97–1,3), *p*=1,0	1,1 (0,63–1,6), *p*=1,0	1,3 (0,99–1,7), *p*=0,45
Calcium, mMol/l	3,3 (3,1–3,6)	5,1 (3,8–6,4), *p*=0,2	4,0 (2,0–6,0), *p*=1,0	3,7 (1,4–6,1), *p*=1,0
Magnesium, mMol/l	2,1 (1,5–2,8)	2,4 (2,1–2,7), *p*=1,0	2,5 (1,4–3,5), *p*=1,0	2,4 (1,9–2,8), *p*=1,0
Diuresis, l^{-3}/day.	13,8 (8,6–19,1)	9,6 (6,5–12,7), *p*=0,3	8,2 (3,9–12,5), *p*=0,18	12,7 (6,1–19,4), *p*=1,0
pH, units of pH	7,8 (7,2–8,3)	6,0 (5,7–6,3)*, *p*=0,0001	6,1 (5,9–6,3)*, *p*=0,00008	6,9 (5,9–7,9), *p*=0,23

Note: * – the differences are statistically credible, $p \leq 0.05$.

Among the biochemical blood serum indicators of the experimental animals we observed the increase in the enzymic activity of ALT in II group by 21.6% and III – by 29.6% compared to control; increase in urea content by 22% is observed in the serum of animals of II experimental group; the level of cholesterol is decreased in the blood of animals of III group and is 63% of control (table 4). In this experiment the decrease in the blood serum cholesterol for rats is not dose-dependent that is why it does not considered as the toxic action indicator.

In relation to the urinoexcretory system we observed the decrease in the value of hydrogen ions on the 30th days of experiment in the urine of animals of II and III groups (table 5) that more likely is associated with change of the acid-base balance induced by HE-ALA.

Conclusions. As a result of conducted experiment it was established that at the repeated epicu-

taneous impact HE-ALA causes low reaction in the skin of white rats in the form of edema. Трансдермальный The transdermal route of HE-ALA intake is characterized by the ALT activity increase, increase in the level of blood serum urea and shift to the acid side of pH in the urine of experimental animals.

The exposure dose of 341 mg/kg is active, with minimal deviations of indicators, and the dose of 75 mg-kg does not result in the changes of condition of the experimental animals during 30 days. Therefore, the dose of 341 mg/kg can be accepted as threshold during the repeated cutaneous impact of HE-ALA.

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