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EXPERIMENTAL SUBSTANTIATION FOR 2-HYDROXYPROPANOIC (LACTIC) ACID TEMPORARY OCCUPATIONAL EXPOSURE STANDARD

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ABSTRACT. *Lactic (2-hydroxypropanoic) acid is an important metabolic component of living organisms. It is also widely used in various industries. Such a wide application of the acid in manufacturing necessitates the regulation of its content in the workplace air. Toxic effects of lactic acid are described in the literature. It was found that 2-hydroxypropanoic acid belongs to hazard level IV by the criterion of acute oral and inhalation toxicity, it causes skin irritation, severe eye damage, has no skin-resorptive or sensitizing effect, does not cause reproductive toxicity and teratogenicity.*
Aim of the Research. *Substantiation for 2-hydroxypropanoic (lactic) acid indicative safe exposure level (ISEL) in the workplace air.*
Methods and Materials. *Analytical, toxicological, statistical.*
Results. *In the process of conducting toxicology study, it was found that in the conditions of inhalation experiment (intranasal modelling) 2-hydroxypropanoic acid causes changes in the state of the nervous system and affects the cellular composition of bronchoalveolar lavage of experimental animals. Therefore, after a single-dose intranasal instillation Limir = Limac, it can be classified as a substance with non-specific irritant effect. It was found that the threshold of a single-dose inhalation exposure is 20 mg/m^3.*
Conclusions. *According to the data obtained in the process of the experiment and data on toxicity parameters and health-based exposure standards of the chemical analogues, the value of ISEL for 2-hydroxypropanoic (lactic) acid in the workplace air was calculated, it is 1.0 mg/m^3, aerosol.*
Key Words: *2-hydroxypropanoic acid, lactic acid, ISEL, workplace air.*

Introduction. Lactic (2-hydroxypropanoic) acid is an organic acid that is an important intermediate metabolite of living organisms, the end product of lactic acid fermentation, a metabolic component and a component of blood and muscle tissue. At the same time, it is widely used in various industries, such as production of food, cosmetics, pharmaceuticals and chemicals. In addition, on the basis of 2-hydroxypropanoic acid a relatively new type of polymeric materials is created – that is a biodegradable plastic [1].

Worldwide demand for lactic acid is estimated at 130–150 thousand tons per year. This product is important to industrial manufacturing, its volume at the world market is estimated at more than 500,000 tons and is increasing rapidly. In the near future it is projected to grow to 20–30% [2].

All above-mentioned factors lead to an increase in the number of workers exposed to lactic acid in the production environment. At the same time, in Ukraine as of 2019, there is no legally approved occupational exposure standard for this chemical compound. Therefore, the development of an indicative safe exposure level (ISEL) of 2-hydroxypropanoic (lactic) acid in the workplace air is a priority.

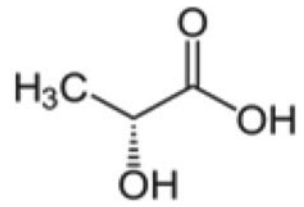
The available literature presents rather complete data on the toxic effects of lactic acid [3, 4, 5, 6]. It was found that 2-hydroxypropanoic acid belongs to hazard level IV by the criterion of acute oral and inhalation toxicity. The median lethal doses of the compound (LD_{50}) when administered orally are: for female rats – 3,200 mg/kg, for male rats – 3,800 mg/kg, for white mice – 4,875 mg/kg, for rabbits – > 5,000 mg/kg. The median lethal concentration of the compound LC_{50} (4 hours, rats) exceeds 7,940 mg/m^3. The clinical picture of acute intoxication is characterized by lethargy, ataxia, respiratory disorders, piloerection, lacrimation, salivation, diarrhea. Percutaneous toxicity studies showed that LD_{50} exceeded 2,000 mg/kg. Lactic acid does not have a skin-resorptive effect. The compound causes irritation of the skin (severe erythema and edema); in an ex vivo experiment, namely with enucleated chicken eye, it causes severe corneal damage, so it is labelled: causes severe eye damage [3]. The compound does not stimulate sensitization of the body in case of contact with the skin; it does not cause reproductive toxicity or teratogenicity [6].

Aim of the Research. Substantiation of the indicative safe exposure level (ISEL) of 2-hydroxypropanoic (lactic) acid in the workplace air.

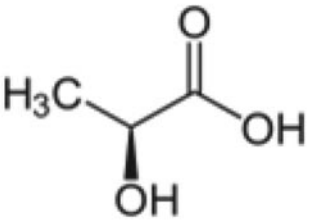
Methods and Materials. International non-proprietary name: lactic acid. Chemical name: α-oxypropionic (2-hydroxypropanoic) acid. Synonyms: 2-Hydroxypropionic acid, DL-Lactic acid, Lactic acid [USP: JAN], Lactic acid, dl-, Propanoic acid, 2-hydroxy-, (RS)-2-Hydroxypropionsaeure, 1-Hydroxyethane-carboxylic acid, Acidum lacticum, Aethyliden-milchsaeure, alpha-Hydroxypropionic acid, Lactate, Lactic acid, Lactic acid (natural), Lactic acid USP, Lacticum acidum, Lactovagan, Milchsaeure, Milk acid, Ordinary lactic acid, Propanoic acid, 2-hydroxy-, Propel, Propionic acid, 2 -hydroxy-, Racemic lactic acid. CAS № 50–21–5 – racemic mixture of L (+) and L (–); 79–33–4 – L (+) α-oxypropionic acid.

Empirical formula: $C_3H_6O_3$.

Structural formula:



(+)-stereoisomere



(–)- stereoisomere

The experimental work was performed on white outbred female rats aged 3–3.5 months and weighing 180–200 g which were kept in the vivarium of Danylo Halytsky Lviv National Medical University. Experimental animals received standard pelleted feed with unrestricted access to drinking water.

The investigational product was administered intranasally in doses corresponding to concentrations of 6 mg/m^3, 20 mg/m^3, 60 mg/m^3. Clinical observations included: monitoring the intensity and nature of motor activity, skin condition, changes in respiration, food and water intake.

A study of the indicators of the state of the nervous system (behavioral reactions and summation-threshold index – STI) was also conducted [7]. The effect on the deep respiratory tract was assessed through citologic analysis of broncho-alveolar lavage (BAL) fluid after a single-dose intranasal instillation [8]. The fluid was obtained by double infusion of the lungs at the rate of 1 ml of saline per 25 g of body weight of the animal. BAL fluid samples were prepared to be analysed and stored by cooling. To calculate the number and quality of bronchial epithelial cells, BAL samples were centrifuged for 10 minutes at 2,000 rotations per minute, the supernatant fluid was poured out, and smears were made from the precipitate and stained according to Romanowski-Geimsa. 200 cells were counted in each smear in the process of microscope observation [9].

During animal studies, the principles of bioethics and legal regulations were followed as well as the requirements of the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [10] and Procedure for Conducting Experiments on Animals by Research Institutions [11].

Results and Discussion. The correlation dependence equation was used to plan studies to establish the acute action threshold (Limac) of 2-hydroxypropanoic acid [12].

$\lg Lim_{ac} = 0{,}76 \lg LC_{50} - 1{,}62$

$\lg Lim_{ac} = 0{,}76 \lg 7940 - 1{,}62$

Lim_{ac} = 21,99 мг/м^3

$\lg Lim_{ac} = 0{,}62 \lg LC_{50} - 1{,}08$

$\lg Lim_{ac} = 0{,}62 \lg 7940 - 1{,}08$

Lim_{ac} = 21,7 мг/м^3

Geometric mean:

< Limac > = **21,8 мг/м^3**

In order to find the acute action threshold (Lim_{ac}) experimentally, lactic acid was administered intranasally in doses corresponding to concentrations of 6 mg/m^3, 20 mg/m^3, 60 mg/m^3. The results of the research prove that all tested concentrations of the preparation do not cause death of experimental animals in.

When animals are exposed to lactic acid at the concentration of 60 mg/m^3, changes in the state of their nervous system are observed (Table 1).

When the compound was instilled at a concentration of 20 mg/m^3, changes in the state of the nervous system were less pronounced and manifested in increased inhibition of the cerebral cortex (as evidenced by the nature of changes in behavioral responses) and nerve impulse transmission (STI change). The lowest

Table 1

Indicators of the nervous system state of white rats after a single-dose inhalation of 2-hydroxypropanoic acid

Indicator	Concentration			
	Control	6 mg/m^3	20 mg/m^3	60 mg/m^3
Summation-threshold index (STI), r.u. (relative units)	3,8 ± 0,2	3,8 ± 0,2	4,1 ± 0,4	5,2 ± 0,3*
Open field test				
Horizontal activity	26,1 ± 3,5	27,6 ± 2,7	17,1 ± 2,1*	15,1 ± 2,6*
Vertical activity	7,5 ± 1,2	6,3 ± 1,4	5,7 ± 1,4	4,1 ± 0,5*
Hole exploratory behaviour	7,3 ± 1,6	6,2 ± 1,3	5,3 ± 0,6	3,1 ± 0,4*
Number of grooming behaviour acts	17,9 ± 5,2	16,2 ± 4,4	13,1 ± 3,9	4,0 ± 1,0*

Note: * – significant differences from other groups ($p \leq 0,05$).

tested concentration did not cause significant changes in the parameters under study.

Intranasal administration of lactic acid to white rats at a concentration of 60 mg/m^3 caused an increase in respiratory rate of experimental animals (165 ± 17,6 breaths / min vs. 122 ± 16,1 breaths / min in control, $p < 0.05$). Simultaneously, there was an increase in the total number of cells in BAL with a decrease in the proportion of macrophages and an increase in the proportion of neutrophils (Table 2). A similar trend was observed when the drug was administered at a dose of 20 mg/m^3, but the changes were insignificant compared to the control, except for the percentage of macrophages (insignificant compared to the control decrease by 13%). When the drug was instilled at a concentration of 6 mg/m^3, the respiratory rate of rats did not differ from the control of reactions, no changes were detected on the part of the cells of the bronchopulmonary segment.

The results of the research prove that the threshold of a single-dose inhalation action is 20 mg/m^3 in terms of macrophages percentage in BAL and horizontal activity in the open field test. The zone of acute action is 397 (hazard level IV). Therefore, since the compound caused simultaneous alterations of the functional state of both respiratory and nervous systems, i. e. the threshold concentrations in terms of general toxicity effects and irritant effect coincide ($Lim_{ir} = Lim_{ac}$), it can be attributed to the substances with non-specific irritant effect.

Table 2

Cellular composition of bronchoalveolar lavage after a single-dose intranasal instillation of 2-hydroxypropanoic acid to white rats

Indicator	Concentration			
	Control	6 mg/m^3	20 mg/m^3	60 mg/m^3
Total number of cells in BAL (10^6)	10,7 ± 1,7	9,50 ± 1,1	8, 9± 0,8	14,04 ± 1,24*
Macrophages, %	90,8 ± 2,8	87,50 ± 5,01	76,9 ± 6,37*	57,5 ± 2,43*
Lymphocytes, %	6,7 ± 2,5	7,6 ± 2,7	8,0 ± 2,5	19,6 ± 1,3*
Neutrophils, %	2,9 ± 0,7	3,9 ± 1,4	4,1 ± 1,8	22,3 ± 1,7*

Note: * – significant differences from other groups ($p \leq 0,05$).

The correlation regression formula was used to calculate ISEL [13, 14].

To calculate ISEL value the following formulas were used:

$lg\ ISEL = 0,92*lg10(Lim_{ir}) - 1,22$ [13]

$ISEL = 0,906\ mg/m^3$

$lg\ ISEL = 0,844*lg(Lim_{ir}) - 1,141$ [14]

$ISEL = 0,948\ mg/m^3$

$ISEL = 1,2 - 0,012*16,8 + lg(M)$ [13]

$ISEL = 2,9\ mg/m^3$

$ISEL = 0,4 - 0,01*M + lg(M)$ [13]

$ISEL = 1,5\ mg/m^3$

The average calculated ISEL value of 2-hydroxypropanoic (lactic) acid in the workplace air is 1.38 mg/m^3.

The recommended ISEL value of 2-hydroxypropanoic acid (lactic acid) in the workplace air is 1.0 mg/m^3, aerosol. In making the calculation the following was considered: lactic acid toxicity parameters, the nature of its biological effect on the body, information on occupational exposure standards of carboxylic acids approved in other countries (Russian Federation, Belarus, Kazakhstan) (maximum permissible concentration (MPC) of citric acid – 1 mg/m^3, succinic acid – 3 mg/m^3), as well as MPC of 2-hydroxypropanoic acid for ambient air approved in the Russian Federation (0.1 mg/m^3).

Conclusions. 1. 2-hydroxypropanoic (lactic) acid acute effect threshold coincides in terms of its general toxicity parameters and signs of irritant effect on the respiratory system and amounts to 20 mg/m^3.

2. The recommended value of 2-hydroxypropanoic (lactic) acid indicative safe exposure level in the workplace is 1.0 mg/m^3, aerosol.

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ЕКСПЕРИМЕНТАЛЬНЕ ОБГРУНТУВАННЯ ТИМЧАСОВОГО ГІГІЄНІЧНОГО РЕГЛАМЕНТУ 2-ГІДРОКСИПРОПАНОВОЇ (МОЛОЧНОЇ) КИСЛОТИ В ПОВІТРІ РОБОЧОЇ ЗОНИ

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РЕЗЮМЕ. *Молочна (2-гідроксипропанова) кислота є важливим метаболічним компонентом живих організмів, а також широко використовується в різних галузях промисловості. Таке широке застосування у виробництві викликає необхідність регламентації її вмісту в повітрі робочої зони. Токсичні ефекти впливу молочної кислоти описані в літературі. Встановлено, що 2-гідроксипропанова кислота за критерієм гострої пероральної та інгаляційної токсичності відноситься до IV класу небезпеки, викликає подразнення шкірних покровів, сильне ушкодження очей, не володіє шкірно-резорбтивною та сенсибілізуючою дією, не спричиняє репродуктивну та тератогенну токсичність.*
Мета. *Обгрунтування орієнтовно безпечного рівня впливу (ОБРВ) 2-гідроксипропанової (молочної) кислоти в повітрі робочої зони.*
Матеріали та методи. *Аналітичні, токсикологічні, статистичні.*
Результати. *За результатами проведених токсикологічних досліджень виявлено, що 2-гідроксипропанова кислота в умовах інгаляційного експерименту (інтраназальне моделювання) викликає як зміни стану нервової системи, так і впливає на клітинний склад бронхоальвеолярного лаважу експериментальних тварин. Отже, після однократного інтраназального введення $Lim_{ir} = Lim_{ac}$, її можна віднести до речовин із неспецифічною подразнюючою дією. Встановлено, що поріг однократного інгаляційного впливу становить 20 мг/м^3.*
Висновок. *За даними, отриманими в експерименті, і даними відносно параметрів токсичності та гігієнічними регламентами хімічних аналогів розраховано величину ОБРВ для 2-гідроксипропанової (молочної) кислоти в повітрі робочої зони, що становить 1,0 мг/м^3, аерозоль.*
Ключові слова: *2-гідроксипропанова кислота, молочна кислота, ОБРВ, повітря робочої зони.*

ЭКСПЕРИМЕНТАЛЬНОЕ ОБОСНОВАНИЕ ВРЕМЕННОГО ГИГИЕНИЧЕСКОГО РЕГЛАМЕНТА 2-ГИДРОКСИПРОПАНОВОЙ (МОЛОЧНОЙ) КИСЛОТИ В ВОЗДУХЕ РАБОЧЕЙ ЗОНЫ

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РЕЗЮМЕ. *Молочная (2-гидроксипропановая) кислота - важный метаболический компонент живых организмов. Она широко применяется в разных отраслях промышленности. Такое разнообразное применение в производстве вызывает необходимость регламентации её содержания в воздухе рабочей зоны. Токсические эффекты воздействия молочной кислоты описаны в литературе. Установлено, что 2-гидроксипропановая кислота по критерию острой пероральной и ингаляционной токсичности относится к IV классу опасности, вызывает раздражение кожных покровов, сильное повреждение глаз, не владеет кожно-резорбтивным и сенсибилизирующим действием, не вызывает репродуктивную и тератогенную токсичность.*
Цель. *Обоснование ориентировочно безопасного уровня воздействия (ОБУВ) 2-гидроксипропановой (молочной) кислоты в воздухе рабочей зоны.*
Материалы и методы. *Аналитические, токсикологические, статистические.*
Результаты. *За результатами проведенных токсикологических исследований выявлено, что 2-гидроксипропановая кислота в условиях ингаляционного эксперимента (интраназальное моделирование) вызывает как изменения состояния нервной системы, так и влияет на клеточный состав бронхоальвеолярного лаважа экспериментальных животных. Таким образом, после однократного интраназального введения $Lim_{ir} = Lim_{ac}$, и ее можно отнести к веществам с неспецифическим раздражающим действием. Установлено, что порог однократного ингаляционного воздействия составляет 20 мг/м^3.*
Вывод. *По данным, полученным в эксперименте, и данным относительно параметров токсичности и гигиеническим регламентам химических аналогов рассчитано величину ОБУВ для 2-гидроксипропановой (молочной) кислоты в воздухе рабочей зоны, которая составляет 1,0 мг/м^3, аэрозоль.*
Ключевые слова: *2-гидроксипропановая кислота, молочная кислота, ОБУВ, воздух рабочей зоны.*

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