TOXICOLOGICAL RESEARCH IN HEALTH RISK ASSESSMENT

UDC 613.63-632: 615.9-099: 612.017.3]: 661.17
DOI: 10.21668/health.risk/2019.2.15.eng

Read online

PECULIARITIES RELATED TO COMBINED EFFECTS PRODUCED BY CHEMICAL ALLERGENS MIXTURE

V.V. Shevlaykov, S.I. Sychyk

Scientific-Practical Hygiene Center, 8 Akademicheskaya Str., Minsk, 220012, Belarus

Workers employed at chemical productions run risks of occupational allergic diseases with chemical etiology. These risks should be assessed and relevant prevention activities are to be substantiated; in this respect it is vital to reveal essence, peculiarities, and mechanisms of hyperimmune response when a body is exposed to a mixture of chemicals with various allergenic and toxic properties. We performed a series of experiments to examine allergenic properties of 57 various chemical mixtures and their separate components; the experiments involved modeling of intradermal introduction in standard doses into albino guinea pigs (ears) or into white mice (bases of tails), the substances were mixed with complete Freund's adjuvant. The obtained results allowed to reveal that intensity of allergenic activity shown by chemical mixtures was determined both by intensity of allergenic properties possessed by specific components in a mixture and by additivity of their immunomodulating effects under combined exposure. These effects naturally depend on a combination of various allergens in a mixture, their contents and quantitative ratios, as well as occurrence of substances with non-specific adjuvant and toxic properties in a mixture. And combined allergenic effects produced by a mixture of chemicals that contains strong chemical allergens in high quantities tend to enhance hyperergic immune response (potentiating) to weaker allergenic components. Predominantly potentiating combined allergenic effects produced by a mixture of chemicals on a body occur due to interrelated mechanisms of specific (elimination of tolerance to conjugate epitopes and modified carrier protein, occurrence of cross-reactive antigen determinants etc.) and non-specific immune modulation (adjuvant, irritating, and immune-toxic effects produced by chemical components in a mixture).

Key words: *a mixture of chemicals, allergens, allergic disease, hyperimmune response mechanisms, essence of allergic processes occurring in a body as a response to exposure to a mixture of chemicals, specific and non-specific immune mechanisms of combined effects produced by chemical allergens.*

Assessment of risks related to occupational allergic diseases (OAD) of chemical etiology, their prevention and hygienic standardization involve a necessity to solve a lot of significant theoretical and practical tasks. One of them is determining essence, peculiarities and mechanisms of hyperimmune response under exposure to a mixture of chemicals (MC) with their allergic and toxic properties having various intensity. Despite its relevance, the issue has been given too little attention over recent years, probably, due to high labor and material costs necessary to perform experimental research.

It is known that when two or more complete or artificial antigens (Ag) are simultaneously introduced into a body, immune modulation between them has intramolecular or intermolecular competitive character; it frequently leads to an immune response to one or several Ag being inhibited and a response to a dominant Ag being restored or inhibited [1]. Chemicals as haptens become complete Ag only after their conjugation with auto-protein carriers that are modified with hapten but still preserve their natural tolerance to a greater or lesser extent [2]. Therefore, immune mechanisms that realize a combined allergenic ef-

Vitalii V. Shevlyakov – Doctor of Medical Sciences, Professor, Chief Researcher at the Industrial Toxicology Laboratory (e-mail: shev-vitaliy@mail.ru; tel.: +375 17 284 13 96, +7 (029) 180-50-26; ORCID: https: //orcid.org/0000-0001-8226-9350).

Sergei I. Sychik – Candidate of Medical Sciences, Associate Professor, Director (e-mail: svkasul@mail.ru; tel.: +375 17 284 03 87, +7 (029) 304-32-11; ORCID: http: //orcid.org/0000-0002-5493-9799).

fect can differ from Ag significantly under simultaneous exposure to several chemical allergens (CA) in MC that happens in real-life environment. It has been confirmed by multiple experimental and clinical data, mostly on enhanced body allergization under simultaneous exposure to several CA. But it still remains unclear what hyperergic immune response develops in a body under exposure to MC depending on a combination of chemicals with different sensitizing activity and toxicity as well as on their fractions in a mixture; possible mechanisms that realize a combined CA effect in a mixture also need to be clarified.

Our research goal was to perform experimental research and detect peculiarities of combined effects produced on a body by CA in a multi-component mixture and to theoretically substantiate possible immune mechanisms that realize it.

Data and methods. We performed experimental research with standardized techniques in order to examine irritating and allergic properties of 57 MC with various composure and their separate components[1]. Sensitization was modeled via intradermal introduction into a ear of an albino Guinea pig in a standard dose equal to 200 μg of MC and their separate components; another way was intradermal introduction into a tail base of a white mouse in a standard dose equal to 100 μg mixed with a surface-active substance (SAS). Essence and intensity of hyperergic immune response was determined via a set of allergy-diagnostic techniques *in vivo* (challenge direct and cross epicutaneous tests, intradermal tests of a swelling in a paw or ear) and *in vitro* (reaction of specific mast cells degranulation, micro-precipitations, blast-cell lymphocytes transformation etc.) that reflected induction of delayed and instant hypersensitivity (DHS or IHS) in a body.

Examined MC and their separate components were assessed as per their allergenic activity and hazard (4 categories overall); to do that, we applied criteria related to DHS intensity and detection, namely frequency of positive integral parameter of challenge intradermal tests in animals from the test group given in scores (in %), validity of discrepancies in average group values in integral test parameters in the test and reference groups as per Student's or Fischer's test, significance levels fixed at $p<0.05$ or $p<0.01$ as per "X" criterion (Van der Waerden criterion).

Results and discussion. Allergic activity of a MC is predominantly determined by strong or intensive allergenic components but sensitizing effects produced by MC are much more intense than detected allergic reactions to cross tests on animals performed with their separate allergic components. Allergy-diagnostic reactions to those components were detected mostly at the same level or a bit lower when they were introduced into animals separately though standard sensitizing doses were 10-100 times higher than their quantitative contents in sensitizing MC doses. Therefore, combined effects produced by MC are determined less by additivity of sensitizing effects produced by separate components than by their mutually modulating interrelations in a body that could have various character depending on sensitizing capacities and quantitative contents in a MC. To prove it, below we describe specific results of examining glass fiver greases (GFG) that contained components with their allergic activity differing in its intensity.

GFG-78 contained strong allergenic components (belonging to the 1st allergic activity category) in equal and quite high quantitative contents, namely TEG-1 epoxy resin and Di-cyan-diamide formaldehyde vinegar (DCV) resin; we detected an inhib-

[1] Requirements to experimental research on examining allergenic properties and substantiating maximum permissible concentrations of chemical allergens in working area air and the atmosphere: methodical guidelines No. 1.1.11-12-5-2003 / V.V. Shevlyakov [et al] / Belarus Public Healthcare Ministry // Collection of official documents on occupational medicine and industrial sanitary / Republican Center for Hygiene, Epidemiology, and Public Health, Republican scientific and Practical Center for Hygiene; edited by V.P. Filonov, S.M. Sokolov. – Minsk: PCHUP "Biznesofset", 2004. – Part 14: Industrial toxicology. – P. 133–156.

ited immune response to the latter component, especially as per DHS, while an intensity of a challenge dermal test showing response to TEG-1 was even a bit higher (average score was equal to 1.7±0.36) than when the component was introduced into animals separately (average score was equal to 1.5±0.4 while its dose was 12.5 times higher) with a simultaneous decrease in a humoral hyperimmune response. Consequently, TEG-1 is a dominant inhibiting immune modulator as regards DCV that is as strong an allergen. However, a bit higher DHS to GFG-78 as a complete mixture (average score is equal to 2.0±0.36) in comparison with its primary allergenic components proves that epoxy resin has a potentiating effect on other, weaker components in the mixture (Massil Ameo, the 3rd allergic activity category). When TEG-1 was replaced in GFG-78 with a weaker allergen TEG-10 (its fraction being the same), it resulted in a decrease in overall allergenic activity of the mixture (average score was equal to 1.7±0.36) against GFG-78, but components had the same immune modulation as the leading allergenic component TEG-10 dominantly inhibited predominantly cell hyperimmune response to another strong allergen DCV simultaneously potentiating sensitizing effects produced by other components.

L.A. Dueva, V.G. Chmut [3] introduced a simple combination of strong chemical allergens, chromium chloride and urea-formaldehyde resin or epoxy (ED-20 + DEG-1 plasticizer), their contents being proportionate, in substantial doses into animals; they also detected mutual reinforcement of a hyperimmune response that was more significant to chromium, that is, they observed a potentiating effect.

If contents of a strong allergenic component are reduced in a mixture, than a component that prevails in its quantitative contents becomes a dominating one as regards inhibiting allergenic effects produced by other components. Thus, epoxy resin ED-5 in GFG-483 inhibited a hyperimmune response to DEG-1 (its contents being 2.5 times lower) mostly as per DHS, but not IHS, with simultaneous reinforcement of effects produced by weaker allergens (KZOT, ES-1) that didn't lead to a substantial decrease in allergenic effects produced by the mixture.

Nevertheless, when a strong or intense chemical allergen is contained in a mixture in low quantity, for example, PR-4 in GFG TZ (0.3%) or PR-3 in GFG-76 (0.15%), and a mixture contains other weaker allergenic components (the 3rd category) in prevailing quantities (PN-M resin in GFG TZ or politerpens in GFG-76), we observed inhibition of a hyperimmune response predominantly as per IHS to a DCV resin, a leading allergenic component, with simultaneous highly active allergic effects produced by PN-M resins and politerpens. Potentiating immune modulations led to high allergization of animals to the whole mixtures GFG-TZ and GFG-76. Similarly, lower contents of DCV leading allergenic component (0.5%) and higher contents of PN-M resin (moderate allergen, 4.0%) in a mixture resulted in mutually enhancing immune modulation. We detected a similar effect when we examined some complex pesticides. Thus, when a strong allergen thiuram D (the 2nd category) was introduced into 53-90 pesticide (up to 14%), it led to an increase in sensitizing activity of another strong allergen, namely Tiabendazole (average score was equal to 0.8±0.27, $p<0.05$ against the control) and the overall mixture (average score was equal to 1.4±0.28, $p<0.01$) against 48-90 pesticide (average score was equal to 0.9±0.24, $p<0.05$). Although Tiabendazole contents were 3 times higher in the latter, average dermal test score in a challenge test performed on animals was lower (0.6±0.13, $p< 0.05$).

A bit different immune modulation in MC was detected when only one strong or apparent chemical allergen was combined with one or several chemicals with weak or moderate allergenic activity. Thus, in spite of DCV component concentration being rather high (2.5%) in GFG TZ-1 and TZ-2,

cellular and humoral hyperimmune response to it was a bit lower than in case of GFG-TZ and GFG-mTZ, but we detected a significant increase in allergenic effects produced by weak allergenic components. such as OkADA, stearox, and oxalen. And when DCV concentrations fell by 2-4 times in GFG-14 and GFG-1k, an immune response to a leading allergenic component became even less intense, especially as regards IHS, and allergy-diagnostic reactions to other components didn't enhance. As a result, detected DHS to the whole mixtures of the above mentioned GFG were significantly lower than to other DCV-containing mixtures.

This mutual weakening of a hyperimmune response was detected under exposure to a mixture of chromium chloride with intense and moderate allergens, for example, naphthalene-formaldehyde resin or divinyl styrene latex SKS-65, while a mixture of the former with a weak allergen (melanin-formaldehyde resin) drastically increased an immune response to the polymer [3]. E.S. Smirnova, G.V. Lomonova [4], S.A. Ashirova et al. [5] obtained similar results reflecting potentiating immune modulation of a strong allergen (toluene diisocyanate) as regards one or two weak sensitizers (dimethyl cyclohexalamine and dimethyl ethanolamine) under combined inhalation exposure to the mixture even at their Lim_{ch} levels. Researchers also detected an enhanced allergenic effect produced by styrene due to a combination with greatly allergenic formaldehyde or similar potentiating of allergenic effects produced by weakly allergenic methyl methacrylate due to strongly allergenic acrylonitrile under inhalation exposure to binary mixtures in comparison with their separate effects at Lim_{ch} levels [6, 7].

At the same time, we didn't detect immune-modulating competitive relations (additive effects) between moderate-weak chemical allergens in MC in intradermal sensitization models regardless of their quantitative contents and ratios in a mixture with relevant relatively weak allergenic activity of the latter (for example, such mixtures as GFG-1, 4-88, 6, 25, Shl-1, 2).

On the other hand, we should take into account influences exerted on immune-modulating relations by allergens and MC components with adjuvant effects and irritating properties. Indeed, when GFG mPE and TZ-1 that didn't contain mineral oils were introduced into animals, we detected not only a decrease in hypersensitivity induction to DCV leading allergen but also less apparent potentiating of effects produced by weaker allergenic components accompanied with a substantial decrease in allergenic activity of these mixtures.

We detected greater allergenic activity of those MC that had more apparent irritating properties. For example, GFG-2 (average score as per local dermal effects is 3.3±0.27) contained DCV in the same quantity as GFG-1k (average score as per local dermal effects is 1.3±0.27) but detected DHS and especially IHS to DCV were 2.5 times higher for the former mixture with simultaneous distinct increase in allergic dermal test-reactions to other components (DBS, ADE-3). Accordingly, GFG-2 had higher allergenic activity (average test-reaction score was 1.9±0.27, $p<0.01$ against the control and the 2nd test) than GFG-1k (average score was 0.75±0.27, $p<0.05$ against the control).

Therefore, allergenic activity of a MC is determined by immune-modulating relations between its components that can induce competitive (inhibiting), independent, or, much more frequently, potentiating hyperimmune response to chemical allergens. Intensity and predominant type of chemical immune modulation mostly depend on a combination of allergens with various intensity, their quantitative contents and ratios, occurrence of substances with non-specific properties in a mixture that influence immune processes. Accordingly, essence and mechanisms of immune modulation that induce allergenic reactions in a body under exposure to a mixture of chemicals with different toxic, irritating, and allergenic effects, will differ from

competition between complete or artificial Ag. And if competitive inhibition of the latter is mostly specific and is similar to immunologic tolerance induction [1, 8], then immune modulation to MC components is implemented via several interrelated and mutually correlated specific and non-specific mechanisms.

In this case specific mechanisms of immune response potentiating are quite similar to immunologic tolerance failure [1, 2, 9, 10]. Let us explain why. Firstly, simultaneous introduction of haptens with different functionally active groups determined their conjugation with different auto-protein carriers, and as T-helpers have non-specific receptors to a carrying section in an Ag, that is, belong to different clones, there is no competition between complex Ag [2, 10].

Secondly, tolerance and, consequently, competitive inhibition of hyperimmune effects is easily eliminated via immunization with cross-reacting Ag [8]. Thus, it was detected in experiments, that immunization of a body with bovine serum albumin (BSA) with two different adjugate groups (acetyl and picrite or arsenilate and sulfanilate) eliminated tolerance to BSA conjugate with one of these haptens. And the higher was an extent of replacement with hapten, the more efficient this conjugate was an agent that eliminated areactivity to a carrier protein [12]. Elimination of tolerance results in occurring anti-bodies and sensitized lymphocytes to new conjugate epitopes and to epitopes of a modified carrier protein and initial auto-protein which tolerance had been to [2, 11].

We should also take into account that haptens immune dominants are specific but a considerable part of ligand dominant created by a modified auto-carrier, especially in case of identical or similar active chemical groups of haptens, has relative specificity [2]. Cross-reacting antigen determinants stimulate activated T-helpers ($CD4^+CD28^+$), which, due to specific and non-specific helper factors, activate b-lymphocytes, macrophages, and effector T-lymphocytes, and it is accompanied with an enhanced antibody and/or cellular hyperimmune response to a combination of haptens [2, 13–15]. Therefore, the stronger haptens modify a protein matrix (that is, have high sensitizing capacities) and the higher their dose is, the more intensely a balance immune regulation mechanism is violated with prevailing IHS reinforcement; on the contrary, weak chemical allergens with preserved tolerance to their carrier auto-proteins will not induce an enhanced hyperimmune response under joint effects.

Indeed, experiment with MC revealed that the stornger allergens a mixture contained, the more intense a potentiating effect was, especially as regards enhanced IHS. With relatively low contents of strong allergens in a mixture, one of them dominantly inhibits predominantly DHS, but not IHS, to a weaker one or that contained in smaller quantity. Simultaneously, other, less active allergens are also activated, mostly as per IHS. If a mixture contains one or even several strong chemical allergens but in low concentrations, it doesn't induce potentiating immune modulation as it is confirmed by data collected by L.A. Dueva et al [16] in their research on combined veterinary medications.

At the same time, non-specific factors make a significant contribution into potentiating of allergenic effects produced by MC. Some research revealed that substances with irritating or adjuvant effects stimulated the macrophage system with adequate activation of cooperation between immune-competent cells and non-specific cytokine regulating network; it led to a greater humoral and cellular immune response to chemical allergens. We should also take into account that multiple industrial chemical mixtures (greasers, cutting lubricants, synthetic detergents, etc.) contain emulsifying and antistatic substances that are mostly synthetic SAS. Many SAS are known to not only have irritating, allergenic, and auto-allergenic properties, but also promote penetration of other chemicals into a body through natural barriers (skin and mucous tunic) [17, 18]. Besides, it has been proven recently, that cation, non-ionic, and especially

poly-cation SAS have apparent dose-dependent activating immune-modulating effects due to activation of macrophages, enhanced migration and cooperation between T- and B-cells with significant stimulation of antibodies formation [1, 19].

It is also quite appropriate to mention violation of cognate identification and idiopathic regulation of an immune response due to immunotoxic effects produced by chemicals on immune-competent cells which are especially sensitive to any metabolic failures, predominantly, on proliferating cytotoxic suppressor T-cells ($CD3^+CD8^+$). This process is significantly involved into potentiating immune modulation of haptens. A deficiency in activated $CD8^+CD28^+$ lymphocytes leads to tolerance failure and is accompanied with polyclonal activation of B-lymphocytes and selection of activated effector T-lymphocytes clones [1, 20, 21].

Therefore, realization of allergic effects by a mixture of chemicals that contains components with different toxic, irritating, and allergenic properties, simultaneously involves multiple interrelated mechanisms of specific immune modulation (elimination of tolerance to epitopes of conjugate and a modified carrier protein, occurrence of cross-reacting antigen determinants, etc.) and non-specific one (adjuvant, irritating, and immunotoxic effects on immune-competent cells) that determine intensity and a predominant type of a hyperergic immune response to a MC and, consequently, a probable clinical course of an OAD.

Conclusion. We analyzed the results obtained in experimental research and it allowed us to make the following conclusions.

1. Intensity of allergenic activity a MC has is determined not only by how intense allergenic properties of its separate components are but also by immune modulating relations between them that can have additive or, more frequently, potentiating effects on a hyperergic immune response.

2. Essence and predominant type of immune modulation effects produced by MC components naturally depend on a combination of allergens with different intensity, their quantitative contents and ratio, occurrence of substances with non-specific adjuvant, irritating, and immunotoxic properties in a mixture:

– if a mixture contains several strong chemical allergens in high concentrations, modulation induces an enhanced immune response between them and to weaker allergenic components;

– if a mixture contains strong and intense chemical allergens, a stronger one or a component that is contained in prevailing quantity dominantly inhibit predominantly DHS of another simultaneously potentiating an immune response mostly as per IHS to other, weaker allergenic components, especially if they are contained in a mixture in high quantities;

– If a strong chemical allergen is contained in a mixture in low quantities, or a mixture contains only moderate and weak allergenic components, a hyperimmune response is additive;

– If a mixture contains chemical allergens and components with adjuvant and/or irritating properties (greasers, SAS, polyelectrolytes), it leads to a significantly enhanced immune response predominantly as per humoral hypersensitivity.

3. Predominantly potentiating effects produced by combined exposure to a mixture of chemical allergens are determined by several interrelated mechanisms of specific and non-specific immune modulation; it should be taken into account when developing and implementing activities aimed at correcting and reducing allergenic activity of MC, their hygienic regulation, and assessing risks related to OAD development in workers and their prevention.

Funding. The research was not granted any sponsor support.

Conflict of interests. The authors state there is no any conflict of interests.

References

1. Petrov R.V. Immunology. Moscow, Meditsina Publ., 1987, 416 p. (in Russian).

2. Alekseeva O.G., Dueva L.A. Allergiya k promyshlennym khimicheskim soedineniyam [Allergy to industrial chemicals]. Moscow, Meditsina Publ., 1978, 270 p. (in Russian).

3. Dueva L.A., Chmut V.G. K voprosu ob immunologicheskoi konkurentsii khimicheskikh allergenov razlichnoi prirody [On immunologic competition by chemical allergens of various nature]. *Sovremennyye problemy gigieny truda i profpatologii v mashinostroenii i khimicheskoi promyshlennosti*: *tez. dokl. resp. konf.* Khar'kov, 1983, pp. 103–104 (in Russian).

4. Smirnova Ye.S., Lomonova G.V. Kombinirovannoe deistvie na organizm khimicheskikh faktorov proizvodstv poliuretanov [Combined effects on a body produced by chemicals factors in polyurethane production]. *Gigiena i okhrana okruzhayushchei sredy v khimicheskoi promyshlennosti: sbornik nauchnyh trudov.* Moscow, 1987, pp. 89–92 (in Russian).

5. Ashirova S.A., Pokrovskaya E.A., Antonyuzhenko V.A., Trifonov V.B. Ozdorovlenie uslovii truda rabotayushchikh v sovremennykh proizvodstvakh penopoliuretanov [Creating healthier working conditions for people employed at contemporary foamed polyurethane productions]. *Gigiena truda i okhrana okruzhayushchei sredy v khimicheskoi promyshlennosti: sbornik nauchnyh trudov.* Moscow, 1987, pp. 19–23 (in Russian).

6. Bogdanov R.V., Sobol' Yu.A., Erm G.I. Allergennye i immunotoksicheskie svoistva smesi formal'degida i stirola [Allergenic and immune-toxic properties of formaldehyde and styrene mixture]. *Zdorov'ye i okruzhayushchaya sreda*. In: S.I. Sychik ed. Minsk, RNMB Publ., 2016, no. 2, pp. 197–200.

7. Vasil'kevich V.M., Erm G.I., Sobol' Yu.A. Osobennosti immunotoksicheskogo i allergennogo deistviya stirola, metilmetakrilata i akrilonitrila pri ikh izolirovannom i kombinirovannom postuplenii v organism [Peculiarities of immune-toxic and allergenic effects produced by styrene, methilmethacrylate, and acrylonytrile under their separate and combined introduction into a body]. *Zdorov'ye i okruzhayushchaya sreda*. In: S.I. Sychik ed. Minsk, 2015, vol. 2, pp. 75–79.

8. Siskind G.U. Immunologicheskaya tolerantnost' [Immunologic tolerance]. *Immunology*. 2-nd edition; In: U. Pol ed. Moscow, Mir Publ., 1988, pp. 362–395 (in Russian).

9. Fontalin L.N., Pevnitskii L.A. Immunologicheskaya tolerantnost' [Immunologic tolerance]. Moscow, Meditsina Publ., 1978, 311 p. (in Russian).

10. Phipps R.P., Roper R.L., Stein S.N. Regulation of B cell tolerance and triggering by macrophages and lymphoid dendritic cell. *Immunological Reviews* 1990, no. 117, pp. 135–158.

11. Schechter J. Antigenic competition between polypeptidil determinants in normal and tolerant rabbits. *The Journal of Experimental Medicine,* 1968, vol. 127, pp. 237–250.

12. Weigle W.O., Dixon F.J. Mechanisms of Hypersensitivity. Little-Brown, Boston Publ., 1973, 529 p.

13. Grin M., Shatten S., Bromberg D.S. Hypersensitivity reaction of delayed type. *Immunology.* 3-rd edition; In: U. Pol ed. Moscow, Mir Publ., 1989, pp. 152–169 (in Russian).

14. Herzenberg L.A., Tokuhisa T., Parks D.R. Epitope-specific regulation. III. A bistable, Ig-resticted regulatory mechanisms central to immunologic memory. *The Journal of Experimental Medicine*, 1981, vol. 155, pp. 1741–1780.

15. Weiss S., Dennert G. T cells lines active in the delayed-type hypersensitivity reaction (DTH). *The Journal of Immunology*, 1981, vol. 126, pp. 2031–2035.

16. Dueva L.A., Khil'ko T.F., Kalinina L.I. K metodologii gigienicheskogo normirovaniya allergennykh smesei postoyannogo sostava (na modeli kombinirovannykh veterinarnykh preparatov) [On methodology for fixing hygienic standards for allergenic mixtures with constant structure (applying a model of combined veterinary preparations)]. *Gigiena truda,* 1990, no. 8, pp. 25–27 (in Russian).

17. Forsling B., Lindberg M., Emilson A. [et al.]. Nickel penetration through skin. Particle probe analyses. *12th International Congress, 12 ICXOM*. Krakow, 1990, vol. 2, pp. 587–591.

18. Schallreuter K.V., Wood J.M. The allergenicity of complex cation. *Biochemical and Biophysical Research Communications*, 1986, no. 1, pp. 221–227.

19. Kasenkov K.U., Shumbalova S.T., Musabayev Ye.ZH., Sundetov ZH.S. Vliyanie PAV s antistaticheskimi svoistvami na nekotorye pokazateli immuniteta [The influence of surfactants with antistatic properties on certain immunity indices]. *Gigiena truda*, 1990, no. 12, pp. 14–16 (in Russian).

20. Fundenberg H., Stites D., Caldwell J., Wells J. Basic and clinical immunology. Los Altos, LMP, 1980, 782 p.

21. Goto M., Mitsuoka A., Sugiyama M., Kitano M. Enhancement of delayed hypersensitivity reaction with varieties of anticancer drugs. A common biological phenomenon. *The Journal of Experimental Medicine*, 1981, vol. 154, pp. 204–209.

Shevlaykov V.V., Sychyk S.I. Peculiarities related to combined effects produced by chemical allergens mixture. Health Risk Analysis, 2019, no. 2, pp. 130–137. DOI: 10.21668/health.risk/2019.2.15.eng

Received: 10.10.2018
Accepted: 14.06.2019
Published: 30.06.2019