

Evaluation of Allergenic, Immunogenic, and Skin-Irritant Effects of Deicing Chemicals in Warm-Blooded Animals

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We studied allergenic, immunogenic, and skin-irritating effects of deicing chemicals HCM (Volgograd), Bishofit (Turkmenia), and Nordix-P (Russia) in model experiments on various types of laboratory animals (rats, mice, rabbits). All the studied deicing chemicals exhibited weak immunotoxic activity and in high doses can cause delayed-type hypersensitivity reaction that manifested in skin irritant effect on the skin and produced a suppressive effect on the humoral immunity. The modified method for assessing skin irritation proposed by us is more sensitive than standard methods.

Key Words: *deicing chemicals; allergenic effect; skin-irritant effect; immunotoxicity; humoral immunity*

The problem of the influence of numerous environmental factors on the human body is now in the focus of many researches worldwide. In megacities, an additional environmental stress and threat to human life is associated with the use of special chemicals, deicing chemicals (DC) for preventing ice-crust of the roads in winter. Safe use of various reagents is a priority problem in Russia and all over the world [3,8,11]. According to the regulatory documents, all DC used in the Russian Federation should have a sanitary and epidemiological certificate that confirms the compliance of production and use of reagents with sanitary norms and rules as well as the safety of the material when used in strict accordance with prescribed rates per 1 m² of surface and technical conditions.

Treatment of highways, streets, driveways, and courtyards with DC is an important safety measure, but at the same time it predetermines a complex of negative effects on the natural environment and, in some cases, on human health [1,2,5,10].

DC are chemically active substances that promote corrosion of metal constructions [13]. Much attention is paid to the effect of DC on the soil and higher plants

[3,4,7]. The effects of DC on soil microbiota and hydrobionts were addressed in some studies. However, published data on the toxicity of DC on warm-blooded animals and humans are extremely scanty. Analysis of the effects of DC on laboratory animals (white rats, mice) in acute and subacute experiments showed that DC used in the streets of megacities are classified as weak toxic substances [2,8,12,14]. At the same time, there are numerous reports in the media on irritant and allergenic effects of DC for pets and humans.

It is known that the immune system is highly sensitive to adverse environmental factors. Pollutants can produce immunotoxic and sensitizing effects thereby causing allergic disease and/or modulating the course of immunodeficient states [15].

Here we evaluate the possible allergenic, immunogenic, and skin irritant effect of DC in a model experiment on different types of laboratory animals.

MATERIALS AND METHODS

We used HCM (Volgograd), Bishofit (Turkmenia), and Nordix-P (Russia) as the most frequently applied groups of DC (Table 1) and 4 types of animals (female BALB/c mice, males white guinea pigs, Chinchilla rabbits, and outbred male rats).

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Possible allergenic effects of DC (HCM, Bishofit, and Nordix-P in doses of $1/_{10}$, $1/_{50}$, and $1/_{250}$ LD₅₀) were evaluated in accordance with Methodical Recommendations for Evaluation of Allergenic Properties of Pharmacological Substances [9]. The study was performed on 30 males white guinea pigs (weighting 200-250 g; Stolbovaya nursery) using the technique of cutaneous applications. The animals were divided into 10 groups (3 rats per group): 3 groups of each DC and one control group (applications of distilled water). The test solutions (or distilled water) were applied on clipped skin (25 cm²) on the side surface of the torso in a volume of 0.5 ml evenly spreading with a spatula. For evaluation of the sensitizing effect of DC, the test substances were repeatedly applied on the skin (daily for 15 days). Then, the animals were kept under standard conditions for 7 days, after which skin scratch test were performed. To this end, two 2-cm² skin areas were clipped on the side opposite to the application site separated by a hair strip in each experimental and control animal. To each animal, 0.1 ml water and 0.1 ml test reagent (in the same doses as during applications) were applied to the prepared skin areas with a 2-ml syringe scratching the skin with the needle. Immediate-type hypersensitivity response was assessed in 1-5, 15, 30, and 60 min. Delayed-type hypersensitivity response was assessed in 24, 48, and 72 h. Weigle scale of anaphylactic activity was used for assessment of possible immediate-type hypersensitivity reaction after allergic tests.

Local skin irritation effects of HCM, Bishofit, and Nordix-P was studied on 42 male guinea pigs of a light color. Only animals with healthy clean skin without mechanical damage were taken in the experiment. The day before the experiment, the hair was carefully clipped on symmetrical sites of the back on both side from the spine leaving a 2-cm hair strip cover between them. The right side served for the application of the test samples and the left side is for the control. Then, 30 and 3% solutions of HCM and Bishofit and native and 1:10 diluted Nordix-P were applied on the prepared skin of guinea pigs; Control areas were treated with distilled water. Each group comprised 6 animals. After the end of exposure, the reagents were removed with warm water and soap. Skin reaction was recorded in 1 and 16 h after the end of single application and then over 2 weeks and compared with the symmetrical (control) area of the same animal. The severity of the erythema was evaluated visually and scored in points; edema was assessed by measuring the skin fold (in mm) using a TR-1-10 thickness gauge.

Local effect of DC on eye mucosa was studied on 9 Chinchilla rabbits (3 rabbits per group). HCM and Bishofit in native form and in 30% aqueous solution

and native Nordix-P were used. After one drop (for liquid reagent) or 50 mg (for solid reagent; particle dispersion up to 10 μ) was applied to the conjunctival eye sac, the nasolachrymal duct was pressed against the inner corner of the eye for 1 min. The reagents were applied once, the animals were followed up over 2 weeks. The damaging effect on the eye mucosa was assessed by the degree of hyperemia and edema and the secretion volume assessed in points.

As during dog walking, DC directly contact with the skin of the paws, the technique for assessing local skin irritant effect of DC was modified and the studied DC were applied to the paws of white mongrel male rats. To this end, the rats ($n=7$) were placed in a cage where organic glass wrapped with a double layer of flannel cloth soaked with the studied DC solutions was put on the cage bottom. The exposure was 2 h (mean time of dog walking during winter). The results of the experiment were recorded immediately after the end of the exposure and in 1 and 16 h after it and then in 2 weeks. Controls were placed in boxes where flannel cloth was soaked with dechlorinated water. The total number of animals in this experiment was 63. In this test, 30, 15, and 3% aqueous solutions of HCM and Bishofit and native Nordix-P and its 1:2 and 1:10 dilutions were used. Skin erythema and edema were assessed visually and scored (in points).

The effect of pollutants on immunogenesis can be assessed by humoral immune response, *i.e.* antibody production in response to exposure to infectious and non-infectious antigens. Possible effect (activation or suppression) of DC on the immune system was assessed by comparing changes in the level of specific IgG in mouse serum in dynamics [6]. Sensitization of laboratory animals (female BALB/c mice) receiving DC in doses of $1/_{10}$, $1/_{50}$, and $1/_{250}$ LD₅₀ was performed against the background of single intraperitoneal injection of BSA (fraction V, 100 μ g/mouse; DIA-M) without adjuvants to all animals. In the experiment, 11 groups (10 mice per group) were used: 9 experimental and 2 control. The mice of control group 1, received BSA injection by the same scheme, the studied reagents were not administered. Control group 2 was intact control. On days 1 (baseline), 7, 21, and 28 days from the beginning of immunization, the levels of specific IgG antibodies in mouse serum were measured by ELISA using monoclonal antibodies to mouse IgG labeled with horseradish peroxidase (Polignost).

Polystyrene plates with high sorption (GosNIImed-polymer) were used in the experiment.

RESULTS

Evaluation of the possible allergenic effect of HCM, Bishofit, and Nordix-P in doses equal to $1/_{10}$, $1/_{50}$, and

TABLE 1. Characteristics and Composition of Analyzed DC

Reagent	Composition	LD ₅₀ , mg/kg
HCM (Volgograd)	mass fraction of CaCl ₂ — 96.5% mass fraction of magnesium calculated for MgCl ₂ — 0.5% mass fraction of other chlorides including MgCl ₂ calculated for ≤1.5% mass fraction of Fe — 0.004% mass fraction of sulfates calculated for sulfate ion — 0.1%	1600±286
Bishofit (Turkmenia)	natural mineral contains up to 97% MgCl ₂ ·6H ₂ O mass fraction of sulfate ions (SO ₄) — 1.1% mass fraction of alkali metal ions Na ⁺ K — 0.8% mass fraction of water insoluble residue — 0.2%	4444.00±956.91
Nordix-P	30-50% solution with inhibitor additive — 1% acetic acid CH ₃ COOH K ₂ CO ₃ KOH glycerol C ₃ H ₅ (OH) ₃	2889.00±478.27

$1/_{250}$ LD₅₀ allowed assessing the risk of delayed type hypersensitivity response in the animals (Table 2).

The irritant effect on the skin of guinea pigs was revealed for HCM in doses of $1/_{10}$ and $1/_{50}$ LD₅₀ (both induced weak reaction), Bishofit in doses of $1/_{10}$ and $1/_{50}$ LD₅₀ (moderate and weak reaction, respectively), and Nordix-P in a dose of $1/_{10}$ LD₅₀ (weakly positive reaction) in 48 h the test, which indicates the possibility of these reagents in the specified doses to cause delayed-type hypersensitivity response.

During the experiments, all animals remained active without body weight loss and visible behavioral abnormalities. All tested DC in the studied doses did not cause systemic anaphylactic reaction of immediate type in sensitized guinea pigs during allergic tests. HCM and Bishofit in doses of $1/_{10}$ and $1/_{50}$ LD₅₀ and Nordix-P in a dose of $1/_{10}$ LD₅₀ have weak allergenic properties that manifested in irritating effect on the skin in guinea pigs. DC in a dose equal to $1/_{250}$ LD₅₀ did not cause delayed-type hypersensitivity in experiments on laboratory animals. Of all tested reagents, Bishofit exhibited the most pronounced and Nordix-P exhibited least pronounced allergenic properties.

Local effect of DC application on the eye mucosa was studied on Chinchilla rabbits over 2 weeks. HCM and Bishofit were used in native form and in 30% aqueous solution and Nordix-P was used in native form (concentration 50 g/liter); each group comprised 3 rabbits. All reagents in native form produced an irritating effect on the eye mucosa. The effect of Bishofit was most pronounced: hyperemia of the conjunctiva (3 points) and eyelid edema (2 points); eye discharge

persisted for 4 days from the start of observations. Nordix-P demonstrated weak irritant effect. HCM and Bishofit in 30% aqueous solution and Nordix-P in a concentration 50 g/liter had no effect on the eye mucosa in rabbits.

Analysis of the effects of PGR on the paws of animals showed that Bishofit (30% aqueous solution) produced a moderate irritating effect, while HCM produced more long-lasting negative effect that persisted for 7 days from the beginning of the experiment. Nordix-P in native form had weak irritating effect persisting for 1 day. HCM and Bishofit (15 and 3% aqueous

TABLE 2. Assessment of Delayed-Type Hypersensitivity in Guinea Pigs in Contact with DC

Group	DC dose, mg/kg	Delayed-type hypersensitivity reaction (points)
Control	—	0
HCM	$1/_{10}$ LD ₅₀	2
	$1/_{50}$ LD ₅₀	2
	$1/_{250}$ LD ₅₀	0
Bishofit	$1/_{10}$ LD ₅₀	3
	$1/_{50}$ LD ₅₀	2
	$1/_{250}$ LD ₅₀	0
Nordix-P	$1/_{10}$ LD ₅₀	1
	$1/_{50}$ LD ₅₀	0
	$1/_{250}$ LD ₅₀	0

solutions) and Nordix-P in concentrations of 50 and 10 g/liter had no negative effect on the paw skin in white rats. The modified method of evaluation of skin irritant effects was most acceptable and sensitive for evaluation of the effect of DC on the skin and mucosae of warm-blooded animals.

Assessment of the modifying effect of DC on immunogenesis (suppression or activation) revealed significant decrease in the studied parameters in comparison with the control. This indicates a decrease in specific IgG as a result of suppression of humoral immune response during exposure to DC.

Thus, the studied DC in the specified doses do not cause systemic anaphylactic reaction in sensitized guinea pigs. All studied reagents in high doses $1/10$ LD₅₀ have a weak allergenic effect and can cause delayed-type hypersensitivity reaction. During evaluation of the local skin irritating effect of DC, the modified method was more sensitive and acceptable compared to standard methods of studying DC, considering the specifics applications of these pollutants. HCM, Bishofit, and Nordix-P had a weak effect on humoral immunity, which manifests in suppression of humoral immune response in experimental animals. HCM produced the most pronounced suppressive effect on the synthesis of specific IgG antibodies.

Definite conclusions about the effect of DC on the immune system of animals can be made after more extensive study of their immunotoxic properties. The inhalation effects of DC and their effect on the respiratory mucosa were not analyzed, though these data are necessary for evaluation of the influence of DC on human health and calculation of their safe concentrations.

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