Annex 9

Standard methods for determining the toxicity of CW agents

For the purpose of classifying super-toxic lethal chemicals, a methodology is proposed for determining their intravenous toxicity in rabbits.

Median lethal doses (LD_{50}), expressed in mg per kg of animal weight, are used for evaluation purposes.

The trials are conducted in laboratory conditions with an air temperature of 18-22°C. Clinically healthy, fully grown animals weighing 2.0-2.5 kg (females and males in a 1:1 ratio) are selected for the experiment.

Each chemical is introduced into the rabbits in a water-acetone or water-alcohol solution. Acetone or alcohol is used to prepare the original mother liquor, which is then diluted with distilled water to produce solutions containing the dose of the tested chemical in 0.05 ml of the solution. 0.05 ml/kg of diluted solution is introduced into the rabbit's auricular vein.

In the first stage of the experiment, an evaluation is made of the dose range within which the median lethal dose of the chemical being studied falls. For this purpose, the substance is administered intravenously to the rabbit in increasing or decresing doses according to the effect observed. The effect is recorded as either "died" or "survived". One rabbit is used for each dose.

After the chemical's toxicity range has been determined, the second stage of the experiment is carried out to determine the value of the median lethal dose. For this purpose four groups of six rabbits are required, three for test purposes and one control group. The test animals are given various doses of the chemical, and the control rabbits an equal amount of solvent.

The results of the intoxication are clinically observed for two days. An autopsy is performed on the animals that have died in order to determine the exact cause of death.

The median lethal dose is calculated by the probit method, which can be carried out either manually, by preparing a logarithmic chart, or on various types of computer, using appropriate programs.

The results indicating the intravenous toxicity of the super-toxic lethal chemicals are entered into a record which shows:

The date and time of the experiments;

Weather conditions;

Data concerning the chemical tested (classification number, place, date and order of selection of samples, external appearance, physico-chemical properties);