Designation: E3002 – 15

Standard Practice for Assessing the Comparative Efficacy of Products Used for the Decontamination of Chemical Warfare Agents (CWAs) on Skin[1]

This standard is issued under the fixed designation E3002; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice establishes an *in-vivo* method for assessing the comparative efficacy of products used for the decontamination of chemical warfare agents (CWAs) on the skin.

1.2 This practice provides a quantitative efficacy comparison of different skin decontamination products.

1.3 To minimize the number of animals used, this *in-vivo* practice should be performed only after rigorous *in-vitro* studies of the candidate decontaminant, which can show the implied claims including chemical neutralization, decontamination studies on surfaces and appropriate testing such as cytotoxicity.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with the use of decontamination products or CWAs. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *Chemical Warfare Agents (CWA), n*—toxic chemicals that have been used as chemical weapons, or have been developed for use as chemical weapons.

2.1.1.1 *Discussion*—The most common chemical warfare agents are: (**1 and 2**):[2] (*a*) nerve agents—tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), VX; and (*b*) blister agents (or vesicants)—mustard and lewisite.

2.1.2 *decontamination, n*—the process of physical removal or chemical neutralization, or both, of CWAs to decrease or prevent health effects due to a dermal contamination.

2.1.3 *in-vitro study, n*—study or protocol performed outside of a living organism, either with or without the use of a biological material.

2.1.4 *in-vivo study, n*—study using a whole living organism.

2.1.5 *Organophosphate Agent (OP), n*—the general name for esters of phosphoric acid that are toxic through inhibition of the enzyme acetylcholinesterase.

2.1.6 *Protective Ratio (PR), n*—the LD_{50} of the decontaminated animals divided by the LD_{50} of the positive control (exposed to CWAs and not decontaminated) animals (**3-5**).

2.1.7 *vesicant agent*—a chemical agent that causes burns and destruction of tissue.

2.2 *Acronyms:*

2.2.1 *GA*—common name: Tabun; IUPAC name: (Ethyl dimethylphosphoramidocyanidate): Organophosphate nerve agent.

2.2.1.1 *Discussion*—This nerve agent is the easiest to manufacture. Consequently, it is more likely that developing countries start their CW arsenal with this nerve agent whereas industrialized countries consider Tabun to be out-of-date and of limited use.

2.2.2 *GB*—common name: Sarin; IUPAC name: ((*RS*)-Propan-2-yl methylphosphonofluoride) Organophosphate nerve agent.

2.2.2.1 *Discussion*—GB is a volatile substance mainly taken up through inhalation.

2.2.3 *GD*—common name: Soman; IUPAC name: (*O*-Pinacolyl methylphosphonofluoridate Organophosphate nerve agent.

2.2.3.1 *Discussion*—A moderately volatile substance which can be taken up by inhalation or skin contact.

2.2.4 *GF*—common name: Cyclohexyl sarin; IUPAC name: (Cyclohexyl methylphosphonofluoridate) Organophosphate nerve agent.

2.2.4.1 *Discussion*—A substance with low volatility which

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[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.

is taken up through skin contact and inhalation of the substance either as a gas or aerosol.

2.2.5 *HD*—common name: Mustard or Distilled Sulfur Mustard; IUPAC name: (bis(2-chloroethyl) sulfide); Vesicant.

2.2.5.1 *Discussion*—In its pure state, mustard agent is colorless and almost odorless.

2.2.6 *L*—common name: Lewisite: IUPAC name: (2-chloroethenylarsonous dichloride). Vesicant.

2.2.7 LD_{50}—a standard measure of toxicity. The individual dose required to kill 50 % of the animals in a test population.

2.2.8 *VX*—common name: VX, IUPAC name: (*O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothioate).

2.2.8.1 *Discussion*—Organophosphate nerve agent, a persistent substance which can remain on material, equipment and terrain for long periods. Update is mainly through the skin but also through inhalation of the substance as a gas or aerosol.

3. Summary of Practice

3.1 Due to the extreme hazards of the chemical warfare agents, the efficacy of decontamination products cannot be evaluated in a human clinical study. This practice has been used to support FDA clearance (for example, RSDL®[3] **(6 and 7)** 510k – K023969) for decontamination devices for use on human skin **(3-5)**.

3.2 Determination of the efficacy of decontamination products for use on the skin against these toxic compounds requires *in-vivo* data, which are more physiologically relevant than *in-vitro* studies. This practice provides a methodology for obtaining comparative *in-vivo* data.

3.3 The practice is used in order to calculate the Protective Ratio of the decontaminant. The Protective Ratio is the LD_{50} of animals treated with the chemical agent and decontaminated, divided by the LD_{50} of control animals (animals treated with the contaminant and not decontaminated) measured 24 hours after exposure to the CWA.

3.4 This practice is based on decontamination efficacy of decontamination products against two nerve agents and one blister agent, for a total of three CWAs. The nerve agents are either G-agents or V-agents based on their chemical structures. The two nerve agents included in the practice are GD (from G-agents) and VX (from V-agents). The blister agent included in this practice is HD.

4. Significance and Use

4.1 This practice specifies an *in-vivo* measurement of CWA decontamination on the skin.

4.2 CWA skin decontaminants will have different modes of action including absorption, adsorption, removal, chemical neutralization or some combination of the above. There is, therefore, no single representative *in-vitro* method for validation of decontamination efficacy of products for skin decontamination. For example, measuring the presence of a radiolabelled chemical warfare agent after chemical neutralization, may give a false positive results. It has been shown that if the agent has been chemically neutralized, the radiolabel may still be present in a non-toxic molecule. In addition, some chemical neutralization methods may break down the original agent, but the breakdown product is highly toxic. In the case of VX, hydrolysis produces a highly toxic product, EA2192 (S-(2-diisopropylaminoethyl) methylphosphonothioic acid **(8)**.

4.3 This standard practice is of significance in that efficacy is thoroughly evaluated to the extent possible to represent use on human skin. *In-vivo* studies have demonstrated that simple chemical monitoring for disappearance of the chemical agent may not be sufficient to measure decontamination and neutralization effectiveness. A standard practice is needed for determining actual decontamination and neutralization by measuring the decrease in mortality or lesion size caused by the agent.

5. Reagents

5.1 All the CWAs for these experiments shall be synthesized in the laboratory where the experiments will be performed or obtained from a legitimate external source which shall be included in the report.

5.2 All test materials shall be at the same temperature as that of the room where the test is conducted at the time of application to the animals.

5.3 Appropriate solvents shall be purchased from legitimate vendors as required and disclosed in the report.

5.4 The standard decontaminant used for comparison should be the product currently accepted for use by the majority of Defense Forces and First Responders in the world, which at this point in time is the RSDL® skin decontamination system.

6. Procedure

6.1 A reputable laboratory animal supplier must be used for any specified species or strain. Only a single constant source of supply for each species should be used by the testing laboratory to maintain genetically homogenous test subjects.

6.2 *Animal Care*—All animal care should conform to the appropriate standards (Association for Assessment and Accreditation of Laboratory Animal Care International AAALAC; http://www.aaalac.org/) and all animal test protocols must have appropriate approvals.

6.3 As required by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), animal care and recording of the information including, but not limited to, the following will be expected:

6.3.1 *Condition of the Animals*—Only animals that show no signs of ill health shall be used for these studies.

6.3.2 *Pre-test Conditioning*—All animals to be used in these studies shall be quarantined for a designated period of time dependent on species and strain and source of the animal to ensure good health prior to use. The period of quarantine should be appropriate for the species and strain of animal. An adequate diet for the species and fresh water shall be allowed

[3] The sole source of supply of RSDL known to the committee at this time is Emergent BioSolutions, 400 Professional Drive, Suite 400, Gaithersburg, MD, 20879. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

with free access. Each animal shall be identified, and food consumption should also be noted. Laboratory animal housing and test environmental conditions shall be maintained according to the acceptable animal care accreditation. Any significant deviations shall be noted and reported.

6.3.3 *Weight of Test Animal*—Pretest weights of the test animal shall be taken five days before and just prior to application of the test material. Only animals that either maintain their weight or show an increase equivalent to others of the same age, sex, and starting weight shall be used for the studies. In the selection of the test subjects an attempt should be made to produce as nearly identical groups as possible.

6.3.4 *Number of Test Animals*—All of the studies designed to measure efficacy of decontaminants need two (2) sets of animals. The first set of animals is used to identify the percutaneous 24 hours LD_{50} for nerve agents or the appropriate dosage range to create measurable lesion sizes. The second set of animals is used to test the comparative efficacy of the decontamination products. For determining LD_{50} values of nerve agents without decontamination, 3 animals per dose, 5 doses per agent will be tested. Thus for the two nerve agents (GD and VX), 3 animals per dose × 5 doses per agent × 2 nerve agents = 30 animals are required. For determining LD_{50} values with decontamination, three stages, 1-2 animals per dose, 4-5 doses will be tested using sequential stage wise methods (**9 and 10**). For the blister agent, 2 sets of animals × 3 animals per set × 1 blister agent = 6 animals required. All test groups should be evenly divided by sex.

6.3.5 *Animal Preparation*—Each animal should be examined 24 hours prior to testing. No animals showing signs of ill health shall be sued for the testing. The experimenter should carefully remove the hair from the backs and sides of the test animals by closely clipping with an electric animal clipper, and take care not to clip closely so as to abrade the skin. Based on preliminary studies or past experience, establish an area on the skin of sufficient size to allow application of the test material.

6.3.6 *Animal Care*—Animals will be received into a vivarium and supplied cage changes, food and water by animal care staff until their day of treatment. Health checks and any monitoring following treatment will be conducted by experimental staff. Animals will be housed individually. Approved standard operating practices (SOPs) will be followed about Receiving Animals into Vivarium, Identification of Animals, Animal Husbandry – Small Animals, Changing and Cleaning of Small Animal Cages, Lab Animal Feeds, and Shaving of Rodents. After 24 hours all test animals will be euthanized using an appropriate method as included in Section 7.

6.4 The animals may undergo an inhalation induction with isoflurane in a carrier gas at appropriate flow rate.

6.5 A patient monitor shall be used to continually monitor animals for physiological parameters such as heart rate, blood pressure, arterial oxygen saturation, respiration rate, and temperature and these parameters shall be recorded.

7. Test Methods

7.1 This practice utilizes the clipped hair guinea pig model (**3-5**). With proper documentation, other animal models with skin similar to human skin, such as hairless guinea pig, rabbit, minipig, or swine, can be used.

7.2 Animals should be anaesthetized during the study. Anesthesia should be appropriate to the animal model selected.

7.3 Exposure to the nerve agents is done by applying a single dose to the application site 5 min after anesthesia. If needed to improve dosing accuracy, diluted agent may be used.

NOTE 1—Use caution when handling nerve agents.

7.4 Exposure to the blister agent is done by applying three doses to three application sites 5 min after anesthesia. If required, to improve dosing accuracy, diluted agent may be used.

NOTE 2—Use caution when handling blister agents.

7.5 Two minutes after agent application, the dosing site shall be untreated (control) or decontaminated (per manufacturer instructions for use) with the test and standard or comparator decontamination product.

7.6 Application method for the decontamination product is to be carefully thought out prior to application to ensure that manufacturer's instructions can be scaled down to the appropriate treatment area.

7.7 Determination of LD_{50} of nerve agents a process which is essentially identification of the dose response curve, should be measured by using doses varying from non-lethal to lethal by establishing 24 hour dose response curves in sets of animals receiving various doses of agent spanning the non-lethal to lethal dose range using sequential stage wise methods (**9 and 10**). Randomly assign the animals to each treatment group and assess mortality at 24 hours. The LD_{50} estimate value is complete when the difference between the upper and lower 95 % confidence limits, divided by the LD_{50} is 0.8 or less.

7.8 To measure decontamination and neutralization efficacy, the study is repeated at agent concentrations starting slightly below the LD_{50} and increasing. The decontaminant products should be used following the commerical usage instructions adapted for the size of the animal, after two minutes of agent exposure. The ratio between the LD_{50} decontaminated and LD_{50} untreated is the protective ratio of the decontaminant. The protective ratio for vesicants is calculated based on decreases in the lesion size, rather than LD_{50}.

7.9 For vesicants, the measurement required is the lesion size in millimetre squared (mm^2) 24 hours after contamination.

7.10 *Monitoring of Nerve Agent Treated Test Subjects to Ensure Minimization of Suffering:*

7.10.1 The treated test subject shall be monitored for signs of OP poisoning for 24 hours following exposure to the nerve agent and decontamination products.

7.10.2 The signs and symptoms to be monitored are: fasciculations, tremors, lacrimation, mouth movement, salivation, vocalizations, abnormal mobility, and abnormal responsiveness.

7.10.3 The subjects shall be rated according to the scale below:

7.10.3.1 *Rating 1*—Normal – no signs.

7.10.3.2 *Rating 2*—One of the listed signs.

7.10.3.3 *Rating 3*—Two or more of the listed signs.

7.10.3.4 *Rating 4*—Any of the listed signs and partial paralysis in either the fore or hind limbs.

7.10.3.5 *Rating 5*—Any of the listed signs, full body paralysis (unable to move hind or front limbs), severely labored breathing, and seizures or convulsions.

7.10.4 Once the test subject has reached a Rating 5, the animal will be humanely euthanized regardless of time after agent exposure.

7.11 *Calculations or Interpretations of Results:*

7.11.1 Efficacy is based on the Calculation of the Protective Ratio.

7.11.2 For the nerve agents, the protective ratio is calculated as the LD_{50} decontaminated/LD_{50} control animals, with LD_{50} measured at 24 h after contamination. To understand the decontamination efficacy of a product, the protective ratio should be compared to the current standard product.

7.11.3 For vesicant agent, the protective ratio is calculated as lesion size millimetre squared (mm^2) of decontaminated animals/lesion size of control (not decontaminated) animals.

8. Report

8.1 All test data and observations shall be documented in a report that includes the following information:

8.1.1 *Test Subjects*—Species and strain, number of animals per dose, sex, weights, physiological parameters, LD_{50} for each CWA tested, and other calculated values, doses administered, toxic signs observed and the onset and duration of such signs.

8.1.2 *Vesicant Agents*—Details of the visual exam of the lesions including size, time post exposure, amount of agent, size of exposure site, and lesion area ratio by treatment and control.

8.1.3 *Test Conditions*—Test material application: undiluted, in simple solution, or in formulation.

8.1.4 *Decontamination*—Actual method and time for decontamination and how manufacturer's instructions were adapted for use on the test subjects.

8.1.5 Time of euthanization post exposure in order to validate hours of observation.

8.1.6 Results of control experiments if conducted.

8.1.7 Results of experiments determining LD_{50}.

8.1.8 Method used for the statistical treatment of the data will be referenced.

9. Keywords

9.1 acetylcholinesterase; Chemical Warfare Agents (CWAs); decontamination and neutralization; GA Tabun; GB Sarin; GD Soman; GF Cyclohexyl Sarin; HD Mustard; *in-vitro*; *in-vivo*; LD_{50}; nerve agents; neutralization; protective ratio; organophosphate (OP); skin; VX Nerve Agent

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