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Report on

CORRELATION OF CONGO-RED-S-328 AND DB-3
TEST PAPERS WITH HUMAN SKIN

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ABSTRACT

This report describes the correlation of the Congo Red-S-328 and DB-3 test papers with skin burns resulting from contact with H and HN contaminated painted metal panels respectively. On the basis of these correlations it was concluded that a painted metal surface which gives a Congo Red-S-328 paper test for H of 45 minutes or greater, or a DB-3 paper test for HN-3 of 12-15 minutes or greater at 35°C, may be classed as safely contaminated in laboratory decontamination tests.

INTRODUCTION

A. Authorization

1. This work was authorized under Bureau of Ships Project No. 397/44. Problems proposed for study were given in Bureau of Ships letter S-S77-2 (Dz) Serial 811 dated 17 December 1940.

B. Statement of the Problem

2. The object of this work was to correlate the chemical tests used in the laboratory to determine the presence of H or HN-3 in painted metal surfaces with physiological reactions caused by such contaminated surfaces when applied to the skin of human subjects.

C. Known Facts Bearing on the Problem

3. Surfaces contaminated with vesicants are a distinct hazard to personnel either from danger of direct contact with the surface or from the vapors evolved from the surface. The contact hazard can be evaluated physiologically by means of patch tests. The vapor hazard is difficult of physiological evaluation because it depends on such factors as temperature and ventilation.

4. In laboratory studies on decontamination it would be desirable to have some simple chemical test which would measure the degree of contamination of different surfaces. By previous correlation with physiological tests it should also indicate whether a surface was safely decontaminated or not.

5. The Congo Red-S-328 test paper, which is an adaptation of the British "Spotted Dick" paper, will indicate the presence of H. The DB-3 test paper will give a test for HN-1, HN-2 or HN-3. In order to interpret the significance of the results obtained using these test papers, it is necessary to determine the reaction on human skin corresponding to a given paper test time.

D. Theoretical Considerations

6. The Congo Red-S-328 paper depends on the reaction of H with the chloroamide to liberate HCl, which changes the Congo Red indicator from red to blue. A sharper color contrast is achieved by impregnating the paper with S-328 only in spots, causing the blue color to develop in spots which stand out more clearly against the original red background. Because the Congo Red-S-328 paper is sensitive to acids, a filter paper impregnated with sodium carbonate is placed between the Congo Red-S-328 paper and the contaminated surface to screen out acid vapors. The freshly prepared Congo Red-S-328 paper has satisfactory sensitivity and gives reproducible results. However, the paper loses sensitivity on standing.

7. The DB-3 detector paper depends on the alkylation of the DB-3 reagent by the nitrogen mustard. The complex first formed is developed by the alkali (sodium carbonate) present in the paper to form a blue compound. The color change of the paper is from white to blue. The contrast is increased by spotting the DB-3 solution on the sodium carbonate impregnated paper, causing the blue color to develop only in these spots.

8. Test paper times are a function of temperature. For this reason the tests are run in a constant temperature box. A temperature of 35°C was chosen because it is close to skin temperature, the paper test times are short and the constant temperature can be maintained without refrigeration.

E. Previous Work Done at this Laboratory

9. The preparation and use of the Congo Red-S-328 test paper is described in NRL Report No. P-1944, "The Use of RH-195 for the Decontamination of HS and M-1," dated 8 October 1942. The DB-3 test paper, as originally developed at this Laboratory, and its preparation and use are described in NRL Memorandum to the Director, "A Laboratory Spot Test for Nitrogen Mustards," dated 13 July 1942. The preparation of the DB-3 test paper, using acetone instead of aqueous methyl cellosolve as the solvent for DB-3 is described in NRL Report No. P-2065, "The Resistance of Impermeable Materials to Penetration by Liquid Vesicant Agents," dated 17 May 1943.

EXPERIMENTAL

Part I. Correlation of Congo Red-S-328 Paper with Physiological Tests for H Contaminated Painted Metal Panels.

A. Procedure

(1) Preparation of Contaminated Panels

10. Painted panels were prepared by brushing one coat of Bureau of Ships Spec. 84-D-1 primer on steel plates, followed by two coats of Bureau of Ships Spec. 20B blue deck paint. These panels were allowed to age at room temperature for several months.

11. A number of three-inch square painted panels were each contaminated with about 0.1 g. of thiodiglycol H. The H was spread with a spatula so that the entire surface was wet with liquid. These panels were allowed to stand at room temperature or were heated in a low temperature oven until the residual contamination, as measured by paper tests, was believed to be approximately that desired. Those panels heated in the oven were allowed to stand at room temperature for at least 12 hours after heating before being used for physiological tests.

(2) Congo Red-S-328 Paper Tests

12. On the day that the physiological tests were to be made, the contaminated panels were tested with freshly prepared Congo Red-S-328 test papers at 35°C and the test time recorded. The first appearance of blue spots was taken to be a positive test. Preparation of the test paper and the method of testing are described in Appendix A. Of the panels tested, six were chosen which gave paper test times of <1, 14, 27, 45, 61 and 80 minutes, respectively.

(3) Physiological Tests

13. As soon as the paper tests were completed for the six panels selected, the panels were cut into 1 cm.² tags. One tag from each of the six panels was taped face down to the flexor surface of the forearms of each of 20 men. A group of 10 men had the tags placed on their forearm in the following order: (The tags are defined by their paper test times.)

<u>Position</u>	<u>Right Forearm</u>	<u>Left Forearm</u>
Top	< 1 min. tag	45 min. tag
Middle	14 min. tag	61 min. tag
Bottom	27 min. tag	80 min. tag

The other ten men had the order of the tags reversed on each arm to compensate for the differences in sensitivity of the skin in the different positions.

14. The tags were worn for one hour. During the wearing period the average temperature was 95°F and the average R. H. was 50%. The men were examined 24, 48 and 72 hours after exposure.

B. Results

15. The physiological results observed at the 48-hour reading are given in Table I. The readings for each man and an explanation of the symbols used are given in Appendix B.

Table I

Physiological Reactions from H Contaminated Tags

<u>Congo Red-S-328</u> <u>Paper Test Time</u> <u>(minutes)</u>	<u>Physiological Reactions</u>						
	<u>AV</u>	<u>V</u>	<u>NPV</u>	<u>E+</u>	<u>E</u>	<u>E-</u>	<u>O</u>
< 1	20						
14		19	1				
27		13	3	4			
45		1	2	4	12	1	
61		2	1	9	8		
80				1	6	12	1

C. Discussion of Results

16. The physiological tests were carried out under severe conditions. The tests were made in June so that the men had "summer skin" and the temperature and R. H. were high during the exposure. Also, the one-hour contact time is longer than would ordinarily be realized. All these factors tend to accentuate the severity of the burns resulting from contact with contaminated surfaces. Normally the contact time with a contaminated surface would probably be less than one hour, the temperature of exposure would

probably be lower and there would not be the intimacy of contact with the contaminated surface because of the clothing worn over most of the body. However, the skin of the arm is not as sensitive as the skin of certain other parts of the body.

17. In view of the severity of the physiological tests it is assumed for the purposes of evaluating this experiment that those tags which did not produce blisters may be considered to be safely decontaminated, as far as contact danger is concerned. The data in Table I indicate that only a small percentage of blisters would result from contact with a contaminated surface if a paper test time of 45 minutes or greater is obtained. Consequently, an H-contaminated painted metal panel giving a Congo Red-S-328 paper test time of 45 minutes or more at 35°C is to be classed as safely decontaminated in laboratory decontamination tests.

Part II. Correlation of DB-3 Paper with Physiological Tests for HN-3 Contaminated Painted Metal Panels.

A. Procedure

(1) Preparation of Contaminated Panels

18. Steel panels painted with blue deck paint were contaminated with HN-3 and partially decontaminated by aeration or gentle heating in the same manner as was done for the H-contaminated panels.

(2) DB-3 Paper Tests

19. The contaminated panels were tested for HN-3 at 35°C by using the DB-3 test paper described in Appendix A. On the day that the physiological tests were to be made, six of these contaminated panels giving paper test times of 3.5, 8, 17, 28, 44 and 58 minutes, respectively, were selected for use.

(3) Physiological Tests

20. As soon as the paper tests were completed for the six panels selected, the panels were cut into 1 cm.² tags. The tags were taped face down to the flexor surface of the forearms of 14 men. Three tags from each panel were worn for 15, 30, 45 and 60 minutes, respectively. The positions of the tags were randomized to compensate

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for differences in skin sensitivity. During the wearing period the average temperature was 85°F and the average R. H. was 60%. The men were examined 24 and 48 hours after exposure.

B. Results

21. The physiological results observed at the 48 hour reading are summarized in Table II. More complete data and an explanation of the symbols used are given in Appendix B.

Table II
Physiological Reactions* from HN-3
Contaminated Tags

Skin Exposure Time (min.)	DB-3 Paper Test Time (min.)					
	<u>3.5</u>	<u>8</u>	<u>17</u>	<u>28</u>	<u>44</u>	<u>58</u>
15	V	O	O	O	O	O
30	V	E	O	O	O	O
45	V	E	E-	E-	E-?	O
60	V	E+	E	E-	E-	O

*Each reaction listed is an average of the reactions on 3 men.

C. Discussion of Results

22. The physiological tests with HN-3 were carried out in September under less severe conditions than were the tests with H, although the men presumably still had "summer skin." The data in Table II show that only mild burns resulted from contact with HN-3 contaminated panels with paper test times of 17 minutes or greater. Consequently, an HN-3 contaminated panel giving a DB-3 paper test time of 12-15 minutes or more at 35°C is to be classed as safely decontaminated in laboratory decontamination tests.

23. It has been shown at this and other laboratories that HN-3 vapor is more vesicant than HN-1 vapor. The burns resulting from contact with partially decontaminated panels are probably caused by vapor and not liquid. Since the DB-3

reagent has about the same sensitivity for HN-1 and HN-3 vapors, it is assumed that an HN-1 contaminated panel giving a DB-3 paper test time of 12-15 minutes or more at 35°C is also to be classed as safely decontaminated.

24. Table II also shows the importance of contact time in producing burns. Thus, an 8-minute panel produced no burns when worn for 15 minutes, but produced papular erythemas when worn 60 minutes.

SUMMARY AND CONCLUSIONS

25. The Congo Red-S-328 test paper has been correlated with skin burns resulting from contact with H contaminated painted metal panels. On the basis of this correlation it was concluded that a painted metal surface which gives a Congo Red-S-328 paper test for H of 45 minutes or greater at 35°C may be classed as safely decontaminated in laboratory decontamination tests.

26. The DB-3 test paper for the nitrogen mustards has been correlated with skin burns resulting from contact with HN-3 contaminated painted metal panels. On the basis of this correlation it was concluded that a painted metal surface which gives a DB-3 paper test for HN-3 of 12-15 minutes or greater at 35°C may be classed as safely decontaminated in laboratory decontamination tests.

RECOMMENDATIONS

27. None. On the basis of these studies, the Congo Red-S-328 and DB-3 paper tests have been used for some time in the laboratory for decontamination studies.

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APPENDIX A

A. Preparation of Detector Papers

1. Congo Red-S-328 Paper. Whatman #44 filter paper is dipped into a 0.03% aqueous solution of Congo Red and allowed to dry. This Congo Red paper is kept in a desiccator at an R. H. of about 50% and may be kept indefinitely. A few minutes before the paper is to be used, it is spotted with a fresh 5% solution of S-328 in tetrachloroethane. A stencil is made by making a number of small holes in a square of cellophane with the point of a pin. The stencil is laid on the Congo Red paper with the projecting sides of the holes downward. A cotton swab moistened with the S-328 solution is rubbed over the cellophane wetting the Congo Red paper in spots. The paper is used as soon as it is dry, because the sensitivity of the paper to H decreases rapidly on standing.
2. DB-3 Paper. Whatman #44 filter paper is dipped into a 10% aqueous solution of sodium carbonate and allowed to dry. This carbonate paper may be kept indefinitely. Shortly before the test is to be made, the carbonate paper is spotted with the DB-3 solution in a manner similar to that described in the preparation of the Congo Red-S-328 paper. The solution is made by dissolving 0.4 g. of pure DB-3 in 10 ml. of acetone. As soon as dry, the paper is ready for use and will give a test for the nitrogen mustards at ordinary temperatures.
3. Sodium Carbonate Paper. Whatman #44 filter paper is dipped into a 10% aqueous solution of sodium carbonate and allowed to dry. This paper may be kept indefinitely.

B. Use of Detector Papers

4. In the tests for H or HN remaining in a contaminated painted metal panel, a piece of sodium carbonate impregnated paper is placed on the panel. The Congo Red-S-328 or DB-3 paper is placed on the carbonate paper and a piece of plate glass is placed over this to hold the papers firmly against the panel. The whole operation is carried out in a constant temperature box maintained at 35°C. The time elapsed to the first appearance of blue spots is recorded as the paper test time.

APPENDIX B

Correlation of Paper Tests with Physiological Tests for H and HN-3 on Painted Metal Panels

Classification of Burns

- O = No reaction
- E-? = Trace
- E- = Mild to moderate erythema
- E = Moderate to severe erythema
- NPV = Numerous pin-point vesicles
- V = Vesicle
- AV = Annular vesicle (with necrotic
center)

Table III

Correlation of Congo Red-S-328 Paper with
Physiological Reactions from Wear of H-Contaminated
Painted Metal Panels

Reactions 48 hrs. after exposure Wearing time - 60 minutes
Temperature - 95°F
R. H. - 50%

Subject	Paper Test Time					
	1 min.	14 min.	27 min.	45 min.	61 min.	80 min.
1	AV	V	NPV	NPV	E+	E
2	AV	V	E+	E	E	E-
3	AV	V	V	E	E+	E+
4	AV	V	V	E+	E	E
5	AV	V	V	E	E+	E
6	AV	V	V	E	E+	E-
7	AV	V	V	E	E	E-
8	AV	V	V	E+	V	E
9	AV	V	V	E-	E+	E-
10	AV	V	V	NPV	E	E-
11	AV	V	V	E+	V	E-
12	AV	V	E+	E	E+	E-
13	AV	V	E+	E	E	E-
14	AV	V	NPV	E+	E+	E-
15	AV	NPV	E+	E	E	E
16	AV	V	NPV	E	E+	E-
17	AV	V	V	E	NPV	E-
18	AV	V	V	V	E+	E
19	AV	V	V	E	E	O
20	AV	V	V	E	E	E-

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