

FOREWORD

This report was initiated by the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory of the 6570th Aerospace Medical Research Laboratories. The contract monitor was Dr. Kenneth C. Back. The original research and development work upon which the report is based was accomplished by Industrial Biology Research and Testing Laboratories, Inc., 22 N. 36th Street, Philadelphia 4, Pennsylvania, under Air Force Contract No. AF 33(657)-8900, in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630201, "Toxicology." The author, Dr. Morris V. Shelanski, was project director in charge of the basic research and development work. Research was begun in October 1962 and completed in January 1964. Mr. Louis Shelanski, Animal Physiologist, and Dr. Theodore Levenson, Chemist, cooperated in the research.

The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

This is the sixth in a series of reports, entitled "Cutaneous Toxicity Evaluation of Air Force Development Materials," by the Industrial Biology Research and Testing Laboratories, Inc. The previous reports are:

- I. WADC TR 56-626, December 1956, by M.V. Shelanski and C. Josephs
- II. WADC TR 57-742, November 1957, by M.V. Shelanski and K.L. Gabriel
- III. WADC TR 59-124, June 1959, by M.V. Shelanski and K.L. Gabriel
- IV. ASD TR 61-77, April 1961, by M.V. Shelanski and K.L. Gabriel
- V. MLR-TDR-62-26, April, by M.V. Shelanski and K.L. Gabriel

Contrails

ABSTRACT

Ten Air Force development materials were studied via the prophetic patch test method on laboratory animals to determine the primary irritant effect, gross sensitization index, and gross percutaneous toxicity of these materials. The patch test studies with rabbits indicated that three of the materials showed very mild primary irritant action. None of the materials produced any sensitization or gross systemic poisoning. Dermal reactions on rabbits were so mild that testing on humans was not precluded. The materials were, therefore, tested on human volunteers by the Shelanski repeated insult patch test. Results of this test indicated that all of the materials were safe to use on contact with human skin.

PUBLICATION REVIEW

This technical documentary report is approved.

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INTRODUCTION

Industrial Biology Research and Testing Laboratories, Inc., was engaged by the United States Air Force to perform dermatological studies and provide cutaneous toxicity data on certain Air Force development materials. These data would serve the Air Force as criteria for establishing safe handling procedures and limits of application of these materials when utilized by Air Force personnel.

There are various methods used for the determination of cutaneous toxicity of a chemical compound or substance. Laboratory animals, such as rabbits or guinea pigs, have been used by many investigators (ref. 1). The true index of cutaneous reaction can, however, only be determined by using human subjects. Prophetic patch tests are one of the methods used for this purpose (refs. 2 & 3). This test method helps to establish both the primary irritation and sensitization characteristics of a compound brought into contact with the human skin. Prophetic patch test studies were performed on laboratory animals to screen the primary irritant and sensitization characteristics of certain Air Force development materials. The Shelanski repeated insult patch test (ref. 3), in addition to giving information about primary irritation and sensitization characteristics of the compound, will also bring out any fatiguing reactions which may occur on continuous contact of the material with the human skin. This technique was performed on volunteer human subjects to define the characteristics of these compounds on the skin of humans.

MATERIALS

The following materials were received from the Aerospace Medical Research Laboratory:

1. Penetrant P-149 2-22-41 MI 2535 C
2. Fluorescent Penetrant Dye FP95
3. VP-30 Visible Penetrant
4. Fluoro-Chek Penetrant
5. Seal-Lock Fluoro Penetrant FD20
6. 1-Chloro-2,2,3,3, -tetrafluoropropane
7. 1-Chloro-2,2,3,3,4,4,5,5-Octafluoropentane
8. 1,1,1,3,3, -Pentachloro-1,2,2, -Trifluoropropane
9. 1-Bromo-2,2,3,3,4,4,5,5, -Octafluoropentane
10. 4-Bromo-1,3 Dichloro-1,2,2,3,4,4-Hexafluorobutane

CRITERIA

FOR GRADING PATCH TEST REACTIONS

The investigators have discussed the criteria for grading patch test reactions used by various authors in a previous report, March 1955 (ref. 4). In this study, as in the previous, the following criteria were used by the Industrial Biology Research and Testing Laboratories, Inc.:

- 0 - no reaction, or questionable reaction
- 1+ - definite or clear-cut erythema
- 2+ - marked erythema, greater than present in 1+ reaction
- 3+ - marked erythema, edema, with or without a few vesicles
- 4+ - marked erythema, edema, with vesicles and oozing

RABBIT SCREENING STUDIES

PROCEDURE

Ten groups of five albino rabbits each were used in this study. The animals selected weighed approximately two kilograms each. Prior to use, the animals were placed on colony diet and observed for a period of two weeks. Animals not showing normal weight gain were replaced.

Prior to patching, the fur on the back of each rabbit was closely clipped to expose an area of skin equal to at least 10% of the total body area. This area was then shaved to denude the skin completely. The patch site area was marked with permanent ink to identify the site for later reference.

The test materials were applied to the denuded skin, covered with glassine paper, and held in place by means of a muslin binder. Approximately four grams of each material was spread over the exposed area of skin for each application. Five rabbits per material were used. The first or primary application remained in contact with the denuded skin for forty-eight hours. Upon removal, reactions were graded and recorded. Twenty-four hours after removal of the patches, the sites were examined for delayed reactions.

Following the primary application, the animals were rested for fourteen days. The patch material was then reapplied on the same site as a challenge or sensitization application. Again, after forty-eight hours contact, the patches were removed and reactions graded and recorded. Twenty-four hours later, the sites were examined for delayed reactions.

RESULTS

Material #1 - Penetrant P-149 2-22-41 MI 2535 C - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #2 - Fluorescent Penetrant Dye FP95 - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #3 - VP-30 Visible Penetrant - produced a 1+ reaction in one rabbit following the removal of the initial application and again following the removal of the challenge application. In both instances the reaction had cleared at the examination 24 hours following the removal of the application.

Material #4 - Fluoro-Chek Penetrant - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #5 - Seal-Lock Fluoro Penetrant FD20 - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #6 - 1-Chloro-2,2,3,3,-tetrafluoropropane - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #7 - 1-Chloro-2,2,3,3,4,4,5,5-Octafluoropentane - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #8 - 1,1,1,3,3,-Pentachloro-1,2,2,-Trifluoropropane - produced a 1+ reaction in all five rabbits following the removal of the initial application and again following the removal of the challenge application. In both instances the reaction had cleared at the examination 24 hours following the removal of the application.

Material #9 - 1-Bromo-2,2,3,3,4,4,5,5,-Octafluoropentane - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material #10 - 4-Bromo-1,3 Dichloro-1,2,2,3,4,4-Hexafluorobutane - produced a 1+ Reaction in all five rabbits following the removal of the initial application and again following the removal of the challenge application. In both instances the reaction had cleared at the examination 24 hours following the removal of the application.

CONCLUSIONS

For the three materials which produced effects, the reactions were not considered sufficient to preclude the human phase of testing.

HUMAN PATCH TESTS

SHELANSKI REPEATED INSULT PATCH TEST

PROCEDURE

Each material was tested on three hundred human volunteer subjects. The sample was applied, using the conventional patch technique, to the skin of the subjects for twenty-four hours and then removed. Skin reactions were graded and recorded. The skin was allowed to recuperate for twenty-four hours. This cycle of contact and recuperation was repeated fifteen times for a total of thirty days, the reaction being graded after each application. Following the removal and the grading of the fifteenth application the skin was allowed to recuperate for two weeks. The material was then re-applied on the same subjects for twenty-four hours. Patches were then removed and the reactions were graded and recorded. The first application gave an index of primary irritation. The final application gave information on sensitization. The repeated applications, from the second through the fifteenth, determined the extent of fatiguing and served to accelerate skin reactions which facilitated forecasting of probability of cutaneous irritation due to long-term exposures.

RESULTS

Material #1 - Penetrant P-149 2-22-41 MI 2535 C - was not a primary irritant or a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #2 - Fluorescent Penetrant Dye FP95 - was not a primary irritant or a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #3 - VP-30 Visible Penetrant - produced a very mild irritant action on the skin of one rabbit and in a single instance an apparent mild irritant action on the skin of one human subject. It produced no fatiguing action or sensitization in any of the 300 subjects tested. No particular significance is attached to the human dermal reaction since it was an isolated response which did not reoccur.

Material #4 - Fluoro-Chek Penetrant - was not a primary irritant or a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #5 - Seal-Lock Fluoro Penetrant FD20 - did not cause any reactions when applied to rabbits' skins. It produced no irritant reactions on the skins of the 300 volunteers. In a single instance it produced a fatiguing action on the skin of a human subject. The intensity of this reaction was no greater, and the numbers reacting much less, than that which would be obtained from a 10% weight/volume aqueous solution of commercially available toilet soap bars when tested under the same conditions. This material did not sensitize any of the 300 human subjects.

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Material #6 - 1-Chloro-2,2,3,3,-tetrafluoropropane - was not a primary irritant nor a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #7 - 1-Chloro-2,2,3,3,4,4,5,5-Octafluoropentane - was not a primary irritant nor a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #8 - 1,1,1,3,3,-Pentachloro-1,2,2,-Trifluoropropane - produced a mild primary irritation in the skins of the five rabbits tested. It was not a primary irritant or a fatiguing agent to the 300 human volunteers. This material did not sensitize any of the subjects.

Material #9 - 1-Bromo-2,2,3,3,4,4,5,5,-Octafluoropentane - was not a primary irritant or a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

Material #10 - 4-Bromo-1,3 Dichloro-1,2,2,3,4,4-Hexafluorobutane - produced a mild primary irritation in the skins of the five rabbits tested. It was not a primary irritant or a fatiguing agent to the 300 human volunteers. This material did not sensitize any of the subjects.

CONCLUSIONS

In this study ten materials produced no significant reactions by either the Schwartz prophetic patch tests on rabbits (ref. 2) or the Shelanski repeated insult patch test on three hundred human volunteers (ref. 3). With respect to sensitization, in using 300 subjects for these tests, we can state with 95% statistical certainty that the materials tested will be a sensitizer to less than 1.2% of the population since none of the 300 subjects showed any sensitizing reactions (ref. 5). These materials may be considered innocuous and may be permitted to contact human skin for prolonged periods.

While all the interpretations and recommendations have been made on the basis of a generally accepted testing procedure, it must be pointed out that the test method is not infallible or above criticism. Further, the patch test situation does not duplicate the range of temperature, humidity, air flow, perspiration, and friction, among other factors, which will be met in actual usage of the material. Because the prophetic patch test was devised to provide screening information with respect to cutaneous irritation and sensitivity from certain materials, it must be emphasized that the test should be used only for that purpose. Therefore, the recommended procedure following the test is to employ the materials within the limits recommended for direct skin contact on a usage basis. This should be done on 5,000 to 10,000 subjects, preferably under variable climatic conditions prior to the release of the materials for general use.

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APPENDIX A

TABLE I

Maximum Intensity Of Reactions On Rabbit Skins

With

VP-30 Visible Penetrant

1,1,1,3,3,-Pentachloro-1,2,2,-Trifluoropropane

4-Bromo-1,3 Dichloro-1,2,2,3,4,4-Hexafluorobutane

<u>Material</u>	<u>Rabbit Number</u>	<u>Primary Application</u>				<u>Challenge Application</u>			
		<u>Day</u>				<u>Day</u>			
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>
VP-30 Visible Penetrant	1	1+	0	0	0	1+	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
1,1,1,3,3,-Pentachloro- 1,2,2,-Trifluoropropane	1	1+	0	0	0	1+	0	0	0
	2	1+	0	0	0	1+	0	0	0
	3	1+	0	0	0	1+	0	0	0
	4	1+	0	0	0	1+	0	0	0
	5	1+	0	0	0	1+	0	0	0
4-Bromo-1,3 Dichloro- 1,2,2,3,4,4-Hexafluorobutane	1	1+	0	0	0	1+	0	0	0
	2	1+	0	0	0	1+	0	0	0
	3	1+	0	0	0	1+	0	0	0
	4	1+	0	0	0	1+	0	0	0
	5	1+	0	0	0	1+	0	0	0

APPENDIX B

TABLE II

Repeated Insult Patch Test

With

VP-30 Visible Penetrant

Number of subjects negative throughout	299
Number of subjects showing 1+ but no higher	0
Number of subjects showing 2+ but no higher	1

<u>Number of Application</u>	<u>Grade of Reactions</u>				
	<u>0</u>	<u>1+</u>	<u>2+</u>	<u>3+</u>	<u>4+</u>
1	300	0	0	0	0
2	299	0	1	0	0
3	300	0	0	0	0
4	300	0	0	0	0
5	300	0	0	0	0
6	300	0	0	0	0
7	300	0	0	0	0
8	300	0	0	0	0
9	300	0	0	0	0
10	300	0	0	0	0
11	300	0	0	0	0
12	300	0	0	0	0
13	300	0	0	0	0
14	300	0	0	0	0
15	300	0	0	0	0
Sub Total	4,499	0	1	0	0
Challenge	300	0	0	0	0
TOTAL	4,799	0	1	0	0

APPENDIX B

TABLE III

Repeated Insult Patch Test

With

Seal-Lock Fluoro Penetrant FD20

Number of subjects negative throughout	299
Number of subjects showing 1+ but no higher	0
Number of subjects showing 2+ but no higher	1

<u>Number of Application</u>	<u>Grade of Reactions</u>				
	<u>0</u>	<u>1+</u>	<u>2+</u>	<u>3+</u>	<u>4+</u>
1	300	0	0	0	0
2	300	0	0	0	0
3	300	0	0	0	0
4	300	0	0	0	0
5	300	0	0	0	0
6	300	0	0	0	0
7	300	0	0	0	0
8	300	0	0	0	0
9	300	0	0	0	0
10	300	0	0	0	0
11	300	0	0	0	0
12	299	0	1	0	0
13	300	0	0	0	0
14	300	0	0	0	0
15	300	0	0	0	0
Sub Total	4,499	0	1	0	0
Challenge	300	0	0	0	0
TOTAL	4,799	0	1	0	0