COMPARISON OF SKIN SENSITIZATION METHODS:
LANDSTEINER, MAGUIRE AND GUINEA PIG MAXIMIZATION

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

GALE D. TAYLOR, Colonel, USAF, BSC
Chief, Toxic Hazards Division

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# Comparison of Skin Sensitization Methods: Interim Technical Report

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## Abstract
Comparison was made of three skin sensitization procedures: Landsteiner, Maguire, and Guinea Pig Maximization. Dinitrochlorobenzene, formaldehyde, and Carbowax 4000 were selected as strong, weak to moderate, and non-sensitizers, respectively. The Maguire Test was most consistent in detecting the effects of these materials in the predicted order. Micropathologic examination of tissue at application sites did not increase the accuracy or precision of the tests.
PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes an Interim Report on the Evaluation of the Landsteiner, the Maguire, and the Guinea Pig Maximization Test Skin Sensitization Methods. The research covered in this report began in February 1980 and was completed August 1980 and was performed in part under Air Force Contract No. F33615-76-C-5005 and F33615-80-C-0512, work unit 63020115. K. C. Back, Ph.D. and M. K. Pinkerton served as the contract technical monitors for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D., served as the Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to R. S. Bowers, E. R. Kinkead, C. L. Gaworski and J. A. Sizemore for their significant contributions and assistance in the preparation of this report.
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INTRODUCTION

A modified form of the Landsteiner-Draize guinea pig sensitization (Landsteiner and Jacobs, 1935; Draize, 1959) method has been used exclusively at the Toxic Hazards Research Unit for a number of years. During the 1977 meeting of the University of California Scientific Advisory Board, it was suggested that some of the newer, more sensitive alternative sensitization procedures be investigated.

The primary criticism of the Landsteiner Test is that it fails to detect many known weak to moderate human sensitizers. Magnusson and Kligman (1969) compared their Guinea Pig Maximization Test (GPMT) with the Landsteiner Test using 24 substances of differing allergenicity. They found that 11 known human allergens failed to sensitize a single animal by the Landsteiner Test while all 11 were readily identified by the GPMT. Klecak (1977) used 32 fragrance materials (reported human sensitizers) in an extensive comparison study of the Open Epicutaneous Test (OET), the Draize modification of the Landsteiner Test, the GPMT, Freund's Complete Adjuvant Test (FCAT), and the Human Maximization Test (HMT). Only 8 of the 32 substances proved positive in the Draize Test. However, 22, 18, and 20 of the 32 materials proved positive in the OET, GPMT, and FCAT, respectively. Numerous other comparison studies have been conducted (Buehler, 1965; Maguire, 1973; Magnusson, 1975; Buehler and Griffith, 1975; Griffith and Buehler, 1977; Magnusson and Kligman, 1970, 1977; Sharp, 1978; Maurer et al., 1979). Almost without exception, each author found his method to be the best for his purposes. Therefore we decided to conduct our own comparison study.

After reviewing the various test methods, the GPMT (Magnusson and Kligman, 1969) and the Maguire Test (Maguire, 1973) were selected. Both these tests appeared to be fairly simple and required no special equipment. Furthermore, the Maguire Test was recommended by a member of the University of California Scientific Advisory Board (private communication, V. K. Rowe, 1978). The GPMT was developed over 10 years ago and is probably the next most well established method after the Landsteiner Test. The Maguire Test, the GPMT, and the Landsteiner represent three different modes of administration in their respective induction phases. The Landsteiner Test uses intradermal injections, the Maguire Test uses topical applications, and the GPMT uses both injections and topical applications.

Three materials of known sensitization potential were selected for test comparison purposes. Dinitrochlorobenzene (DNCB) was selected as a severe sensitizer (Klecak et al. 1977). Formaldehyde was chosen as a known weak to moderate sensitizer (Magnusson & Kligman, 1969). Carbowax 4000 (Carpenter et al., 1971) was selected as the nonsensitizer.
Evidence exists that sensitizing agents can be quantitatively identified by measuring the degree of mononuclear cell infiltration in the dermis of exposed animals (Groth, 1978). Therefore, histologic evaluation of the skin was included in this study to investigate the applicability of inclusion of this technique as a part of our routine screening procedures.

MATERIALS AND METHODS

Test Materials

Dinitrochlorobenzene (practical grade, Lot No. DIT30), formaldehyde (Reagent Grade 37%, Lot No. 5108), and Carbowax 4000 (polyethylene glycol 4000) powder (laboratory grade, Lot No. 763795) were obtained from Fisher Scientific Company.

The concentrations used were based on the results of preliminary primary irritation tests. However, the maximum concentration was limited to 0.1% in the case of the Landsteiner protocol. Due to some unexpected results in the first trials, second trials of the GPMT and Maguire Tests with formaldehyde were conducted to determine the effect of concentration. Sodium lauryl sulfate, a primary irritant, was utilized in the second GPMT in an attempt to increase the sensitivity of that test.

Animals

Hartley strain, female albino guinea pigs 6 to 8 weeks of age obtained from Murphy Breeding Laboratory, Plainfield, Indiana were used for all studies. Test groups initially consisted of 20 animals for all test methods.

After visual evaluation of the animals, skin sections were taken from the application sites of all animals for histologic evaluation. Cross sections of the skin sections were viewed under the light microscope.

Landsteiner Test

Before beginning sensitization tests, three guinea pigs were used to determine the primary irritation properties of each material as the Landsteiner Test required the use of non-irritating concentrations. For this purpose, 0.05 and 0.1 ml quantities of 0.1% solutions (maximum allowed for this method) or suspensions of the materials in the proper vehicle were injected intradermally into the closely clipped scapular and sacral areas of three guinea pigs. Distilled water was used as the vehicle for Carbowax and formaldehyde while 1% acetone in peanut oil was used with DNCB.
Similar injections of the vehicles alone were made. The test material injection sites were compared with vehicle injection sites at 24 and 48 hours postinjection.

The sensitization tests were started on a Monday. The guinea pigs were weighed and closely clipped on the scapular areas. A volume of 0.05 ml of a 0.1% solution of the test materials was injected intradermally into the upper right scapular area of each guinea pig. A similar injection of the vehicle was made concurrently into the upper left scapular area. Readings were made 24 and 48 hours later and recorded on the sensitization record sheets.

Doses of 0.1 ml of the freshly prepared 0.1% solution were injected into clipped dorsal lumbo-sacral areas of the guinea pigs on the following Wednesday, Friday, Monday, etc., until seven doses were administered insuring that new skin sites were selected for each injection.

The guinea pigs were rested for three weeks, weighed and given a challenge dose of 0.05 ml of the test material solution in the lower right scapular area. A control injection of the vehicle alone was also administered into the lower left scapular area. The reactions were read after 24 and 48 hours.

The grading system was designed so that the intensity of the skin reaction was represented by a proportionate numerical value and any reaction elicited by the vehicle was subtracted from the reaction elicited by the test material and vehicle combined.

The product of the width and length of the wheal (in mm) was multiplied by the following reaction scores:

- 0 = needle puncture ("np") - no wheal
- 1 = very faint pink ("vfp") - no value for this reaction
- 2 = faint pink ("fp")
- 3 = pink ("p")
- 4 = red ("r")
- 5 = bright red ("R")
- 6 = edema - <1 mm in height ("e")
- 7 = edema - >1 mm in height ("E")
- *8 = necrosis - <1 sq. mm ("n")
- *9 = necrosis - >1 sq. mm ("N")

* The product of width and length of the necrotic area multiplied by 8 or 9 was added to the numerical value of the foregoing reactions that were present - calculated in the same manner.
A final grade of 25 or less indicated no sensitizing potential, a final grade of 100 indicated a moderate sensitization potential, and a score of greater than 200 indicated a severe sensitization potential.

**Maguire Test**

The materials were tested for primary irritation on 3 guinea pigs by application to the clipped flank. Observations were made at 24 hours for signs of irritation. If the test material was irritating to the guinea pig skin, further dilution was made until the non-irritating concentration was reached. The maximum non-irritating concentration was used in the sensitization test.

An area on the back of each animal directly above the forelegs was clipped with electric clippers. The area was then chemically depilated with a wet paste mixture of 50% barium sulfide and 50% Tide® detergent (by weight) on the morning of the first insult exposure. Test solutions, 0.1 ml for each application, were applied to these areas on 1/2 x 1/2 inch cotton gauze squares, covered with dental dam, and held in place with elastic adhesive tape. The first application remained in place for two days. It was then removed and a second application of 0.1 ml was made. Two days later, this patch was removed, a total of 0.2 ml of Freund's Complete* Adjuvant (FCA) per animal was injected intradermally, using 2 points adjacent to the insult site, and a new patch of 0.1 ml of the test material was then applied. On the third day after this application, the patch was removed and a fresh patch of 0.1 ml of the material applied. The last patch was removed two days later, and the animals were allowed to rest for two weeks. Each time the patches were removed, the condition of the skin at the application site was examined, evaluated, and recorded. After the last patch was removed, the toes of the hind feet of each animal were taped to prevent the animal from scratching the irritated area. About 1 1/2 to 2 inches of 1/2 inch adhesive tape was wrapped around each foot so that no toenails protruded. The tape was replaced as it was lost.

After the two-week rest period, both flanks of the animals were clipped and challenged on one side with the same test solution (freshly prepared) as that used during the sensitizing period. The vehicle was applied to the other flank. The challenge applications were not occluded. The skin response at these sites was recorded at 24 and 48 hours after application. Any animals showing greater erythema and/or edema at the test solution challenge site than the

* Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.
vehicle site were rated as positive responders. Skin reactions were evaluated by the method of Draize (1959).

**Guinea Pig Maximization Test (GPMT)**

Five groups of animals were used in the GPMT, e.g., three experimental groups and two control groups (water and ethanol). Ethanol was used as the vehicle for DNCB, water for Carbowax and formaldehyde.

Intradermal and topical applications were utilized with the test material injected independently as well as incorporated in FCA. Three guinea pigs per test material were used to determine the appropriate injection and topical test material concentrations. The concentrations of the test agents were adjusted to the highest levels that could be tolerated locally and generally.

In the experimental groups on day zero, an area of 4 x 6 cm on the shoulder region was clipped with an electric clipper. Three pairs of intradermal (id) injections were made concurrently so that on each side of the midline there was a row of three injections. The injection sites were just within the boundaries of a 2 x 4 cm area to be covered by a patch one week after injection. Paired injections were: (1) 0.1 ml FCA alone (adjuvant blended with equal amount of water); (2) 0.1 ml test material; and (3) 0.1 ml test material in FCA. Injections (1) and (2) were given close to each other and nearest to the head; injection (3), most caudally.

The control groups were shaved, depilated and injected in the same manner without inclusion of the test material as follows: (1) 0.1 ml FCA alone (adjuvant blended with equal amount of water); (2) 0.1 ml vehicle alone; and (3) 0.1 ml vehicle in FCA.

Seven days later, the same area over the shoulder region was again clipped and shaved with a safety razor. The test agents were applied to a 2 x 4 cm filter paper patch to saturation. The patch was placed over the injection sites and covered by an overlapping layer of dental dam. This, in turn, was firmly secured by an elastic adhesive bandage and wound around the torso of the animal. The dressing remained in place for 48 hours. The control animals were exposed to the vehicle without the test agent in the same manner as the experimental group.

After a 14-day incubation period, both experimental and control animals were challenged in the same way. Areas (3 x 3 cm) on both flanks were clipped and shaved. Occlusive patches (2 x 2 cm) were applied for 24 hours. The patch on the left side was saturated with the test agent while the patch on the right side was saturated with the vehicle.
Patch sites were evaluated at 24 and 48 hours after the challenge application. Erythema was defined as redness ranging from scattered and mild to intense and swollen.

Test sites were compared with vehicle control sites to evaluate the sensitization potential. Once again, control site reactions were subtracted from test site reactions.

In scoring the GPMT, the important statistic was frequency of the reaction rather than intensity.

The following table was used to classify test materials as to sensitization potential.

<table>
<thead>
<tr>
<th>Sensitization Rate (%)</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-8</td>
<td>I</td>
</tr>
<tr>
<td>9-28</td>
<td>II</td>
</tr>
<tr>
<td>29-64</td>
<td>III</td>
</tr>
<tr>
<td>65-80</td>
<td>IV</td>
</tr>
<tr>
<td>81-100</td>
<td>V</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Sensitization Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Weak</td>
<td>0-8</td>
</tr>
<tr>
<td>II Mild</td>
<td>9-28</td>
</tr>
<tr>
<td>III Moderate</td>
<td>29-64</td>
</tr>
<tr>
<td>IV Strong</td>
<td>65-80</td>
</tr>
<tr>
<td>V Extreme</td>
<td>81-100</td>
</tr>
</tbody>
</table>

**Pathologic Examination**

After the 48 hour evaluation, all animals were sacrificed and tissue sections were taken from treated and control sites for micropathologic evaluation of inflammatory and cellular infiltration reactions.

Microscopic examination of the skin sections was performed by Lt Col A. Hall, III. The following parameters were evaluated microscopically on a scale of 1 to 4 with 4 being the most severe:

1. Mononuclear inflammatory reaction
2. Acute inflammatory reaction
3. Hyperemia
4. Spongiosis
5. Acantholysis
6. Bullae
7. Eosinophilic infiltration
8. Scabs
9. Epithelial Necrosis
10. Dermal Edema
11. Vacuoles in Dermis
12. Scar

In addition to the monocyte severity evaluation, actual counts of monocytes were made.
RESULTS

The results of the gross evaluation (Table 1) confirmed that the Landsteiner Test identified dinitrochlorobenzene (DNCB) as a strong sensitizer. The mean reaction scores at 24 and 48 hours were 653 and 403, respectively. Ninety and 75% of the exposed animals demonstrated positive results, respectively. The mean reaction scores and percent of positive responders in the formaldehyde and carbowax trials did not indicate definite sensitization reactions. Landsteiner scores of these two materials, one a weak to moderate sensitizer and the other a non-sensitizer, were essentially equal.

<table>
<thead>
<tr>
<th>Test Materials</th>
<th>Carbowax 4000</th>
<th>Formaldehyde Test #1</th>
<th>Formaldehyde Test #2</th>
<th>DNCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>Peanut Oi</td>
</tr>
<tr>
<td>Mean Reaction Scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 24 hrs.</td>
<td>44</td>
<td>51</td>
<td>0</td>
<td>653</td>
</tr>
<tr>
<td>at 48 hrs.</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>40</td>
<td>403</td>
</tr>
<tr>
<td>Positive Responders, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 24 hrs.</td>
<td>20</td>
<td>15</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>at 48 hrs.</td>
<td>5</td>
<td>0</td>
<td>32</td>
<td>75</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only 1 Animal.

The results for the gross evaluations of the Maguire trials are summarized in Table 2. The results confirmed that DNCB was a potent sensitizer and Carbowax 4000 was a nonsensitizer. Two trials were conducted with formaldehyde at different concentrations. This was necessary because 37% formaldehyde proved to be corrosive to the skin of the guinea pig when applied using a patch and held covered by dental dam. The results from the trial in which the higher concentration of formaldehyde was used indicated that formaldehyde was a potent sensitizing agent. However, formaldehyde appeared to be a weak sensitizer when tested as a 5% aqueous solution, a non-irritating concentration.
TABLE 2. SENSITIZATION POTENTIAL OF TEST MATERIALS USING MAGUIRE PROCEDURE

<table>
<thead>
<tr>
<th>Test Materials</th>
<th>Carbowax 4000</th>
<th>Formaldehyde Test #1</th>
<th>Formaldehyde Test #2</th>
<th>DNBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (%)</td>
<td>10</td>
<td>37-10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Acetone</td>
</tr>
<tr>
<td>Positive Responders, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 24 hrs.</td>
<td>0</td>
<td>89</td>
<td>21</td>
<td>76</td>
</tr>
<tr>
<td>at 48 hrs.</td>
<td>0</td>
<td>84</td>
<td>5</td>
<td>71</td>
</tr>
</tbody>
</table>

<sup>a</sup> 37% used on first application, 10% used thereafter.

The results of the gross evaluation of the GPMT are summarized in Table 3. DNBC had a strong sensitization potential and Carbowax 4000 demonstrated no sensitization reaction. Formaldehyde trials were conducted with 2 concentrations of the injected induction dose. In the first trial, 0.1% formaldehyde in water demonstrated no sensitization potential. A mild sensitization reaction was elicited in the 2nd trial using 0.2% formaldehyde concentration.

TABLE 3. SENSITIZATION POTENTIAL OF TEST MATERIALS USING GUINEA PIG MAXIMIZATION TEST PROCEDURE

<table>
<thead>
<tr>
<th>Test Materials</th>
<th>Carbowax 4000</th>
<th>Formaldehyde Test #1</th>
<th>Formaldehyde Test #2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DNBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. Inj., %</td>
<td>5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Conc. Top., %</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Peanut Oil</td>
</tr>
<tr>
<td>Positive Responders, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 24 hrs.</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>95</td>
</tr>
<tr>
<td>at 48 hrs.</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sodium lauryl sulfate was applied to the animals 24 hours prior to the topical application.
Analysis of monocyte infiltration and other micropathologic measurements of inflammatory reactions at the challenge sites indicated that there was no consistent correlation between any of the parameters examined and the severity of immune reactions. This was true in each of the techniques used for evaluation of skin sensitization. Therefore, we decided that under the conditions of this study micropathologic examination of skin sections contributed nothing to determination of the sensitization potential of the three test chemicals.

CONCLUSIONS

By gross observation, the known sensitization potential of dinitrochlorobenzene was confirmed by the Landsteiner Test. The mean reaction scores of formaldehyde (Trials 1 and 2) and Carbowax 4000 were quite low and essentially equal. If these are interpreted as negligible responses, then the known sensitization potential of formaldehyde was not confirmed. However, if the scores are interpreted as positive sensitization responses, the known non-sensitizing capacity of Carbowax 4000 was not confirmed, leading to a false positive conclusion.

The known sensitization potentials of all test materials were confirmed by gross observation in the Maguire Test. The difference in the percentage of positive responders in the formaldehyde trials indicated that the method was concentration dependent. The Maguire Test gave the most consistent results for predicting the known sensitization potentials of the 3 materials.

Dinitrochlorobenzene was confirmed as a strong sensitizer while Carbowax 4000 was a non-sensitizer by the GPMT. None of the animals demonstrated a positive response to formaldehyde in the first trial. Increasing the concentration of the injections and inducing primary irritation prior to the application of the topical induction patch in the second trial appeared to increase the sensitivity of the test, as 25% of the animals responded. This would indicate formaldehyde to be a sensitizer.

For reliable results, care must be taken in the selection of concentrations for the Maguire Test and GPMT. Incorrect concentrations were initially selected in the Maguire Test because the primary irritation tests were conducted as uncovered topical applications. This was done because the challenge applications were to be uncovered. However, the 37% concentration of formaldehyde found to be non-irritating when applied uncovered caused severe damage when applied on a patch and covered with dental dam during the sensitization phase. The concentration was reduced to 10% for the remaining applications. In the second Maguire formaldehyde study, the concentration was reduced to 5%. In the GPMT, the injected formaldehyde concentration used in the second trial was greater than that of the first trial as the results of the first trial were
not in agreement with the findings of Magnusson (1969). Also, sodium lauryl sulfate was applied prior to the induction patch to induce primary irritation in an attempt to increase the sensitivity of the test.

The use of only 3 test materials limits the reliability of a comparative test. However, the results of gross observations appear to support the criticism of the Landsteiner Test, i.e., it failed to detect the known sensitization potential of formaldehyde. It is possible that increasing the percentage of the test material, formaldehyde, might increase the sensitivity of the test, as it did in the Maguire and Guinea Pig Maximization Test. However, increasing the test concentration is sometimes impractical because of irritation resulting from intradermal injection of the test material.

The Landsteiner Test requires over 5 weeks from start to finish while the Maguire and GPMT require only a little more than 3 weeks. However, in practice, the Landsteiner Test requires the least man hours to perform, followed by the Maguire Test and the GPMT. It takes considerably less time to perform an intradermal injection than to prepare and apply a patch.

Of these 3 sensitization methods, the Maguire Test appears to have the most advantages. It gave the most consistent results, required less total time than the Landsteiner Test to perform and only slightly more man hours. Dermal sensitization tests performed at the THRU will use the Maguire Test in the future.
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Buehler, E. V. (1965), Delayed contact hypersensitivity in the guinea pig, Arch. Derm., 91:171.


