Experimental Studies on Skin Hazard with 'Versatic' 9-II Acid and its Monoglycidyl and Vinyl Esters

C. G. HUNTER, V. K. BROWN, and L. W. FERRIGAN

From 'Shell' Research Limited, Tunstall Laboratory, Sittingbourne, Kent

'Versatic' 9-II is a mixture of tertiary carboxylic acids each having 9 to 11 carbon atoms in the molecule. 'Versatic' 9-II and its monoglycidyl and monovinyl esters are used in the paint and resin industries.

The vinyl ester of 'Versatic' 9-II has been shown to possess skin irritating and sensitizing properties comparable to those of butyl and 2-ethylhexyl acrylates.

'Versatic' 9-II and its monoglycidyl esters are much less irritant than the vinyl ester and should not prove hazardous to the skin, but unnecessary contact should be avoided.

'Versatic' is the registered Shell trade mark for a series of tertiary aliphatic carboxylic acids. To specify the particular acid, the number of carbon atoms present is used as a suffix, hence the simplest member of the series, 'Versatic' 5, is trimethylacetic acid, and in Fig. 1a the $R_2$, $R_3$, and $R_4$ are methyl groups for this acid.

This paper is limited to derivatives of the material described as 'Versatic' 9-II, which is a mixture consisting principally of tertiary carboxylic acids each possessing a total of nine to 11 carbon atoms and with highly branched and fully saturated chains. In addition to the acids, the monoglycidyl esters (Fig. 1b) and the monovinyl esters (Fig. 1c) have applications in the paint and resin industries.

Because of the likelihood of skin contact an investigation into the potential skin hazard associated with these products was considered desirable. In the United Kingdom, however, there are no official methods for evaluating the skin injury associated with the use of materials, and it was necessary to develop a test programme based on methods described in the literature.

For comparison, two esters of acrylic acid (Fig. 2a) were included in the programme, butyl acrylate (Fig. 2b) and 2-ethylhexyl acrylate (Fig. 2c). These materials have some properties and uses in common...
with ‘Versatic’ 9-11 and its derivatives, and there is well-documented experience with this type of compound (Malten and Zielhuis, 1964).

Methods

The programme of tests was designed to investigate skin sensitization and primary irritation effects using laboratory animals.

Sensitization The development of an eczematous sensitization requires two major factors, an allergen and a genetically appropriate host (Rostenberg, 1960), although the latter is less important in man than in the laboratory animal (Calnan, 1963a). The choice of species for testing is limited to the guinea-pig or the mouse (Crowle and Crowle, 1961). Other species are generally too insensitive or impractical in use. Most laboratories would also regard mice as too insensitive for routine use.

For use in this investigation the ‘P’ strain of guinea-pig (bred at Tunstall Laboratory) was used. This has been shown to be sensitized by a number of different organic compounds of low molecular weight injected intradermally or applied to the skin surface in a non-sensitizing solvent at a concentration of 0.1% w/v. The test was carried out by injecting or applying the solution to the shaved skin on the backs of the guinea-pigs on three days (Mondays, Wednesdays, and Fridays) in each of three successive weeks. The animals then received no treatment for 10 days and a ‘challenge’ dose of the same solution on the right flank and of solvent on the left flank on the eleventh day. Following the ‘challenge’ the animals were examined at one hour, 24 hours, and 48 hours for signs of a sensitization-type reaction, i.e., intense redness, wealing, or both.

Subjective assessments were made of the intensity and duration of the reaction. These, together with the proportion of animals responding in each group, were taken to give a measure of the ‘sensitization potential’ of the product.

In the event of a negative result, the whole test was repeated using the same guinea-pigs.

Primary Irritation Dermatitis due to primary irritants results from direct damage of skin cells and is not mediated by an antigen-antibody reaction (Calnan, 1963b). It is sometimes stated that a primary irritant produces overt signs of irritation after a single exposure. However, this is not necessarily true, and delayed primary irritant effects can only be observed in some cases after prolonged or repeated exposure (Rostenberg, 1962), this being called ‘skin fatigue’.

The choice of experimental animals is difficult as no species has a skin comparable with that of man. The pig might have been considered a reasonable choice (Parish, 1960), but doubt has been expressed on the analogy between pig and human skin (Montagna and Yun, 1964). It was decided to use four species: the rabbit, because of its popularity in some countries (Draize, 1959); the guinea-pig, because of its ‘mosaic pattern’ of hair growth (Flesch, 1954); the hairless mouse (Homburger, Tregier, Baker, and Crooker, 1961); and the formaldehyde-treated rat (Finkelstein, Laden, and Miechowski, 1963).

The tests were of two types, those in which the skin was exposed to the materials inside an impermeable covering, and those in which the skin was treated with free access to the atmosphere. Each material was subjected to the following tests.

(1) Two male and two female albino rabbits (New Zealand White breed, bred at Tunstall Laboratory) were used. The dorsal hair between the shoulders and the hindquarters was closely shorn by means of fine electric clippers. On the first, second, and third days of the test the rabbits were immobilized for periods of six hours in a special holding device. Patches of lint, about 2 cm. x 2 cm., were cut, and 1 ml. of test material was applied to each. Two patches were laid on each rabbit’s back and covered with a sheet of thin polythene; these were bandaged into position by means of a 5 cm. open-weave bandage.

A visual assessment of the degree of erythema and oedema was made using the classification shown in Table I. Notes were made of any other gross changes. Seven days after the first application the final visual assessment was made, and specimens of the animals’ skins were taken for histopathological examination and rating as shown in Table II.

(2) One male and one female rabbit (New Zealand White breed) were shorn as before on the Monday of each week of the test. Daily, five days per week for four and one-half weeks, 1 ml. of the test material was dropped onto the shorn area near the midline. A daily visual assessment was made of the gross skin damage.

On the day after the 23rd application of test

### TABLE I

<table>
<thead>
<tr>
<th>Degree of Erythema</th>
<th>Erythema Score</th>
<th>Degree of Oedema</th>
<th>Oedema Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No erythema</td>
<td>0</td>
<td>No oedema</td>
<td>0</td>
</tr>
<tr>
<td>Pale pink</td>
<td>1</td>
<td>Soft skin</td>
<td>1</td>
</tr>
<tr>
<td>Redness</td>
<td>2</td>
<td>Oedema</td>
<td>2</td>
</tr>
<tr>
<td>Severe redness</td>
<td>3</td>
<td>More definite oedema</td>
<td>3</td>
</tr>
<tr>
<td>Beet redness</td>
<td>4</td>
<td>Severe oedema</td>
<td>4</td>
</tr>
</tbody>
</table>

...
TABLE II
CLASSIFICATION OF ACUTE CONTACT DERMATITIS

<table>
<thead>
<tr>
<th></th>
<th>Evidence of slight spongiosis and acanthosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Spongiosis, acanthosis, and keratosis with slight inflammatory reaction of superficial dermis</td>
</tr>
<tr>
<td>++</td>
<td>Spongiosis (vesicle formation), acanthosis, parakeratosis, and inflammation of dermis</td>
</tr>
<tr>
<td>+++</td>
<td>Necrosis of epidermis, ulceration, and marked inflammation of dermis</td>
</tr>
</tbody>
</table>

material, the skin was removed for histopathological examination and classification as shown in Table II.

(3) Test 2 was repeated on five male and five female albino guinea-pigs ('P' strain, bred at Tunstall Laboratory) using 0.5 ml. of test material each time.

(4) Two female hairless mice (CAH-strain, bred at Tunstall Laboratory) were used. Undiluted test materials were painted onto the ventral skin on 12 consecutive days by means of fine paint brushes. A visual assessment of the skin damage was made daily.

(5) Ten albino rats (Porton-SPF strain, bred at Tunstall Laboratory) were used. Each rat was anesthetized with an initial dose of 40 mg./kg. pentobarbitone sodium, administered intraperitoneally, and anaesthesia was maintained with further doses of the barbiturate administered subcutaneously (20 mg./kg. every two hours). Each rat was tied onto a board with its ventral surface exposed. Hair was shorn from the abdomen by means of fine electric clippers. Undiluted formalin was painted onto the shorn surface three times at half-hourly intervals, the area being allowed to dry spontaneously before five lint squares, each 1 sq. cm., were placed on the abdomen. Test material, 0.2 ml., was placed onto each piece of lint. In each test each rat was exposed to all five materials, the relative positions of which were varied from rat to rat. The lint was held in place with a single strip of polythene sheeting fastened across the rat by means of pins on either side. A subcutaneous injection of 1 ml. 0.5% w/v aqueous trypan blue was made in the axilla. After six hours the polythene and lint were removed and the exposed areas were examined for blueing, a visual assessment of the relative coloration being recorded.

Results

Sensitization Aqueous suspensions containing 0.1% w/v of the test materials in intradermal and topical tests gave the results shown in Table III.

TABLE III
SENSITIZATION REACTIONS OBSERVED IN GUINEA-PIGS

<table>
<thead>
<tr>
<th>Material under Test as 0.1% w/v Aqueous Suspension</th>
<th>No. of Guinea-pigs showing Positive Sensitization Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Topical Test</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>Butyl acrylate</td>
<td>10/10</td>
</tr>
<tr>
<td>2-Ethylhexyl acrylate</td>
<td>10/10</td>
</tr>
<tr>
<td>'Versatic' 9-11</td>
<td>0/10</td>
</tr>
<tr>
<td>Vinyl ester of 'Versatic' 9-11</td>
<td>10/10</td>
</tr>
<tr>
<td>Glycidyl ester of 'Versatic' 9-11</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Two materials, 'Versatic' 9-11 and its monoglycidyl ester, produced no signs of a sensitization reaction. Both acrylates and the vinyl ester of 'Versatic' 9-11 produced signs of sensitization, i.e., intense redness and oedema. It was not possible to rank their sensitization potential since all were about equal.

Primary Irritation (1) The results on rabbits with occlusive dressings over the exposure sites are summarized in Table IV. Both acrylates were more irritating than the 'Versatic' 9-11 derivatives, but the vinyl 'Versatic' ester was capable of this test of

TABLE IV
SUMMARY OF RESPONSES OBTAINED AFTER THREE DAILY EXPOSURES TO THE TEST MATERIALS USING OCCLUSIVE DRESSINGS ON RABBITS

<table>
<thead>
<tr>
<th>Materials</th>
<th>Total Assessment for Four Rabbits*</th>
<th>Histopathological Assessment†</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyl acrylate</td>
<td>152</td>
<td>12</td>
<td>+++</td>
</tr>
<tr>
<td>2-Ethylhexyl acrylate</td>
<td>116</td>
<td>2</td>
<td>+++</td>
</tr>
<tr>
<td>'Versatic' 9-11 acid</td>
<td>53</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>Vinyl ester of 'Versatic' 9-11</td>
<td>100</td>
<td>0</td>
<td>+++</td>
</tr>
<tr>
<td>Glycidyl ester of 'Versatic' 9-11</td>
<td>93</td>
<td>0</td>
<td>++</td>
</tr>
</tbody>
</table>

*Score key—Table I. †Score key—Table II.
causing considerable irritation, and this was rather more persistent than that caused by the acrylates.

(2) Applied daily to the skin of rabbits all five materials soon caused effects. In every case erythema developed but was most severe with the two acrylates and the vinyl ‘Versatic’ ester; with the acid and the glycidyl ester the erythema faded considerably between successive applications and was only severe for the first hour after each application. Epidermal thickening and sloughing were apparent in rabbits treated with the acrylate and vinyl ‘Versatic’ ester. After 12 days foci of necrosis were apparent in the rabbits exposed to the acrylates so these experiments were then terminated, whereas the rest were continued to the twenty-third application without necrosis appearing. Table V summarizes the data obtained in this test.

(3) Results with guinea-pigs were very similar to those for rabbits except that the responses to the acrylates were slightly more severe. A summary of these results is also given in Table V.

(4) Signs of irritation were seen on mice with butyl acrylate and vinyl ‘Versatic’ ester but not with the other materials. The appearance of the irritated animals was one of hyperexcitability immediately following the application and a localized erythematous condition lasting about one hour.

(5) In the tests on rats, blueing was only detectable in three patches on each rat, and these corresponded to the two acrylates and vinyl ‘Versatic’ ester. From the intensity of the blue colour the most affected was seen to be butyl acrylate, and the vinyl ‘Versatic’ ester was about as irritant as 2-ethylhexyl acrylate.

**Discussion and Conclusions**

Acrylates in general are known to be irritants and sensitizing to the skin (Sax, 1963), and butyl acrylate has been described as slightly irritant to rabbit skin, whereas 2-ethylhexyl acrylate has been described as a moderately severe irritant (Fassett, 1963). In our experiments butyl acrylate was undoubtedly more irritant than the 2-ethylhexyl acrylate, although we would predict only a marginal difference under conditions of normal use.

We are not aware of any authenticated cases of dermatitis due to derivatives of ‘Versatic’ 9-11. From these animal experiments we would expect vinyl ‘Versatic’ ester to prove no more dermatotoxic than butyl or 2-ethylhexyl acrylate. ‘Versatic’ 9-11 and its monoglycidyl ester can be expected to be considerably less irritant to the skin than the acrylates, but, in our opinion, precautions should be taken to avoid any unnecessary skin contact.

**REFERENCES**


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