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A comparison of predictive irritation tests with surfactants on human and animal skin

V. K. H. BROWN*

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Synopsis—Nine DETERGENT base materials have been examined in a series of *in vivo* and *in vitro* TESTS involving the use of HUMAN SKIN and various ANIMAL SKINS. Lack of agreement between the results from different tests was apparent and a cautious approach to predictive SKIN-IRRITANCY testing with detergents is advocated.

INTRODUCTION

As part of an investigation into the toxicology of surfactants used in the manufacture of detergents for domestic and other purposes, it was decided to compare the results of some predictive skin irritancy tests using animal and human skin. For the purposes of these investigations neither skin sensitization reactions nor irritation of the eyes were included.

The human skin studies were carried out by Smeenk (1, 2) and the animal studies by the author.

EXPERIMENTAL

Materials

A list of the materials used is shown in *Table I* together with the pH

*Tunstall Laboratory, Shell Research Ltd., Sittingbourne, Kent.

Table I

Surfactants used in skin irritancy investigations

Reference code used in this and other publications(1-4)	Materials	
	Description (pH of 1% w/v aqueous solution)	Structure
T1	Na salt of alkylbenzene sulphonate in which the alkyl chain contains 12 carbon atoms derived from propylene tetramer and is highly branched (pH 6.9)	C_nH_{2n+1}-C$_6$H$_4$-SO_3Na, n=12
T2	Na salt of alkylbenzene sulphonate in which the alkyl chain is essentially unbranched and contains 10-13 carbon atoms, derived from olefins produced by cracking of urea-extracted wax (pH 7.2)	C_nH_{2n+1}-C$_6$H$_4$-SO_3Na, n=10-13
T3	*iso*-Octyl phenol condensed with 8-9 mole ethylene oxide (pH 6.5)	*iso*-C_8H_{17}-C$_6$H$_4$-O-$(CH_2$-CH_2-$O)_nH$; n=8-9
T4	*iso*-Octyl phenol condensed with 15 mole ethylene oxide (pH 6.5)	*iso*-C_8H_{17}-C$_6$H$_4$-O-$(CH_2$-CH_2-$O)_nH$; n=15
T5	Sodium secondary alkyl sulphate with 8-18 carbon atoms in alkyl chain (pH 8.1)	$C_nH_{2n+1}OSO_3Na$; n=8-18
T6	Pure fatty acid soap (pH 10.2)	$C_nH_{2n+1}COONa$; n=12-18 (mostly 12 and 14)
T7	Na salt of sulphated broad-cut coconut ethoxy (3EO) alcohol (pH 6.0)	C_nH_{2n+1}-$(O$-CH_2-$CH_2)_3$-OSO_3Na; n=mostly 12 and 14
T8	Broad cut coconut dimethylamine oxide (pH 6.0)	C_nH_{2n+1}-$N(CH_3)_2O$; n=12 (+ some 14)
T9	Na salt of sulphated broad-cut coconut alcohol (pH 9.7)	$C_nH_{2n+1}OSO_3Na$; n=mostly 12 and 14

values for 1% w/v aqueous solutions. The same batches of surfactants were used for the human and animal experiments.

Methods

Human skin

The methods used for both *in vivo* and *in vitro* tests have been described in detail elsewhere (1-4). These tests included:–

1. *In vivo* tests
   - 1.1. Arm and hand immersion. Tests carried out using 0.1% and 1% aqueous dilutions of active material. Results assessed by clinical observation and histological examination of skin biopsy samples.
   - 1.2. Patch tests. Results based on a clinical scoring system. A 1% aqueous dilution of active material used throughout.
2. *In vitro* tests
   - 2.1. Epidermal water-binding capacity.
   - 2.2. Water-detergent epidermal component extractability.
   - 2.3. Epidermal keratin denaturation.
   - 2.4. Permeability of epidermis to potassium ions and to the detergents.

Animal skin

The tests were of two main types:–

a. Those in which rabbits and rats were exposed to the materials beneath an impermeable covering, thus simulating the conditions which exist when materials get inside protective clothing (e.g. rubber gloves). Each material was therefore subjected to the following tests:

Method 1

Four male and four female albino rabbits were used for each test. 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 6 h in a special holding device. Patches of lint, about 2 cm × 2 cm, were cut, and 1 ml of 0.1% w/v or 1% w/v aqueous active material was applied to each. Two patches were laid on each rabbit's back, covered with a sheet of thin polyethylene and bandaged into position by means of a 50 mm open-weave bandage.

A visual assessment of the degree of erythema and oedema was made using scoring systems ranging from 0 to 4 for the degrees of both erythema

and oedema, intravenous sulphan blue being used as an aid to assessment (5). 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 these findings incorporated in the irritancy rating.

Method 2

Young adult male albino rats were anaesthetized with urethane administered intraperitoneally. Each rat was tied on to a board with its abdomen exposed. Hair was shorn from the ventral surface by means of fine electric clippers, then undiluted formalin was painted onto the shorn surface three times at half-hourly intervals. After the area had dried spontaneously, lint squares, each 1 cm^2, were placed on the abdomen and 0.2 ml of 5% w/v aqueous active material was placed on to each piece of lint. The lint was held in place with a single strip of polyethylene sheeting fastened across the rat by means of pins on either side of the rat. A subcutaneous injection of 0.5 ml of 1% w/v aqueous trypan blue was made in the axilla. After 16 h the polyethylene and lint were removed and the exposed areas were examined for blueing, a visual assessment of the relative coloration being recorded.

b. In the other type of test, uncovered skin of rabbits, "hairless" mice and guinea-pigs was treated for prolonged periods, these being more representative of the exposure of skin during normal working conditions.

Method 3

For each product, one male and one female albino rabbit were shorn on the Monday of each week. Daily (five days per week) for four and one-half weeks (i.e. 23 applications), 1 ml of 1% w/v active test material in aqueous solution was dropped on to the shorn area near the midline. A daily visual assessment was made of the gross skin damage.

On the day after the last application of test material, skin was removed from each animal for histopathological examination.

Method 4

Method 3 was repeated (i.e. 23 applications in four and one-half weeks) on five male and five female albino guinea-pigs, the dose in this case being 0.5 ml of 1% w/v active test material in aqueous solution each time.

Method 5

Each of the surfactants under test was painted on to the dorsal skin of young adult "hairless" mice as a 2% w/v active material aqueous solution. The paintings were carried out daily (seven days per week) for either one or four weeks. For each group the controls were painted, in an exactly comparable manner, with distilled water.

On either the 8th or 29th day, the mice were killed by cervical dislocation and the dorsal skin removed for histological and histochemical examination or for oxygen uptake manometry. The histochemical tests used are shown in *Table II*, and oxygen uptake was used as an index of general metabolic change (6).

Table II

Histochemical changes in "hairless" mouse skin induced by painting with 2% w/v active material aqueous solutions of test materials for 28 consecutive days*

+ = increased activity — =no change

Histochemical stain for activity	Surfactant								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
Phosphogluconate dehydrogenase	—	—	—	+	—	—	—	+	—
Glucose-6-phosphate dehydrogenase	+	—	—	+	—	—	—	+	—
Alkaline phosphatase	—	—	—	—	—	—	—	—	—
Acid phosphatase	—	—	—	—	—	—	—	—	—
Monoamine oxidase	—	—	—	+	—	—	—	+	—
Succinic dehydrogenase	+	—	—	+	—	—	—	—	—
DNA	+	—	—	—	—	—	—	—	—

*Comparable mice exposed for seven consecutive days showed no changes in any of the histochemical tests.

Results

For the purposes of this investigation, the results of all the individual tests have been assembled such that the irritancy can be ranked according to the method used. Assessments based on gross and histological changes are shown in *Table III*, and those based on chemical methods in *Table IV*.

The results of the human skin studies exhibited reasonable statistical agreement within each investigation (1). Because of their biological homogeneity there was little variation between animals of one species in the tests dependent on subjective assessment of morphological changes. More variation was observed in the histochemical assessment and in the oxygen uptake by skin (6).

Table III

Ranking of skin irritation results with human and animal skins
(Gross and/or histological examination)

Detergent key - *Table I.*

Irritancy rating	Covered exposures				Uncovered exposures			
	Human patch tests	Rabbits (Method 1)		Rats (Method 2)	Human arm-immersion tests	Rabbits (Method 3)	Guineapig (Method 4)	"Hairless" mice (Method 5)
		1% w/v	0.1% w/v					
LEAST IRRITANT	T3 T4 T7	T3 T4	T1 T2 T3 T4 T5 T6	T3 T4 T6 T7 T8 T9	T3 T6	T1 T2 T3 T4 T5 T6 T9	T1 T2 T3 T4 T5 T6 T9	T2 T3 T6 T9
	T1 T2 T5	T5 T6	T7 T8 T9	T5	T1			
					T5	T7	T7	T1 T5 T7
		T1 T7		T1	T9	T8	T8	T8 T4
	T9	T8			T8			
	T6	T2		T2				
MOST IRRITANT	T8	T9						
Not tested					T2 T4 T7			

Table IV

Ranking of skin irritation results with human and animal skins
(Chemical methods)

Detergent key – *Table I*

Irritancy rating	Animal skin		Human skin			
	"Hairless" mouse		Amino-acid extraction	Measurement or -SH groups	Measurement of permeability to	
	Histochemical change	Oxygen uptake			K+ ions	Detergent
LEAST IRRIANT	T2 T3 T5 T6 T7 T9	No correlation established (6)		T3 T4 T7 T8	T3 T4	T1 T3 T5 T7 T9
				T6	T1 T5 T7 T9	
	T1 T4 T8		T1 T3 T5	T5	T2	
				T9	T8	T6
MOST IRRITANT			T6 T9	T1 T2	T6	
Not tested		T5 T6 T7 T8 T9	T2 T4 T7 T8			T2 T4 T8

Studies on reversibility of effect were not included in these investigations.

Discussion and conclusions

It is immediately apparent that there is poor concordance in the results obtained by the different methods. For example, T9 fluctuates between most irritant, intermediate and least irritant for both human skin and animal skin. Indeed, Smeenk (2) has concluded that the *in vitro* tests he used with human skin and the patch tests carried out with human volunteers were not suitable for screening the irritancy of detergents on the skin. This conclusion presupposes that the arm immersion test is the method to give the most meaningful results but it is well-known that many factors (e.g. size of test population, representativeness of the test group in relation to the population "at risk", ambient climatic conditions, etc.) affect the outcome of arm immersion tests (7-9).

In a detailed report on a wider range of chemicals (10) it has been suggested that the guinea-pig might serve as a useful alternative to the rabbit in the assessment of skin irritancy, but it is our opinion, based on a much more varied range of chemicals than are reported here, that the guinea-pig and the rabbit tests are complementary rather than alternative to each other. However, given the same test conditions, there were no apparent differences in the reactivity of rabbit and guinea-pig skin with these surfactants. There was noticeably good agreement between the results obtained with occluded patches on formalin treated rat skin and those on untreated rabbit skin. However, in our laboratory we have been less successful in discriminating between a series of concentrations of sodium lauryl sulphate than were the originators of the formalin treated rat skin method (11, 12).

The skin of "hairless" mice behaved in an anomalous manner under the influence of the nonionic detergent T4. Using histochemical methods three dehydrogenase systems were stimulated (*Table II*). Monoamine oxidase activity was also increased by T4. The only other surfactant in this series with an effect on monoamine oxidase was T8 and this is less surprising because the surfactant is a derivative of dimethylamine and a latent reserve of monoamine oxidase is known to be present in mouse skin (13). The lack of correlation between oxygen uptake *in vitro* and exposure of "hairless" mouse skin to these surfactants has already been reported (6).

A heterogeneous group of chemicals such as surfactants may react with skin in many different ways (14). Given different conditions of exposure the same chemical may behave in a different manner in contact with the skin

(e.g. T6, a soap famed for its "mildness" to skin, was at the low end of the irritancy scale in uncovered tests in humans and animals but in the medium irritancy grade in patch tests, and in the most irritant grade in some of the biochemical tests). In reality, the skin is seldom abused by a surfactant alone; the presence of fillers, perfumes and other components may modify the irritancy potential substantially [e.g. T8 was found to be among the most consistently irritant materials in this series and yet other laboratories have shown (15), and we have confirmed (unpublished results), that formulations of T8 with some added anionic surfactant are less irritant than is T8 alone]; however, an analysis of the chemical and other factors involved are clearly outside the scope of these investigations.

From these data we conclude that preliminary predictive tests of the type described using animals are at least as useful as comparable tests in humans. If appropriate, human studies should be undertaken after preliminary animal experimentation, but these human studies must be on an adequate scale and statistically designed to be meaningful (16).

In the long term, studies on the biochemical changes brought about by surfactant-skin interactions will aid understanding of the aetiology of detergent dermatitis. Currently such studies do not offer much hope for simplified predictive testing.

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