

Review Article

Determination of skin irritation potential in the human 4-h patch test

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Recently adopted legislation in Europe has increased the focus that must be placed on the development of *in vitro* alternatives to the traditional toxicology tests employed to identify the human health hazards associated with chemicals. Included in these is the rabbit skin-irritation test which is used to discriminate those substances which possess significant acute skin irritation potential from those which are of more limited irritation potential. So far, the considerable efforts to replace this assay with *in vitro* alternatives have not been successful, which may in part be due to the relatively poor quality of the existing *in vivo* dataset. To help address this problem, we have elected to present our complete database of information on the skin irritation potential of some 65 substances, all of which have been tested in a standard human 4-h patch test. These provide a high quality dataset, generated in man (the goal of toxicologists' health protection-related activities). The data are presented in the context of results with a concurrent positive skin irritation control to ensure that results from individual experiments can be correlated. Consequently, *in vitro* or *in silico* alternatives which can identify the significant acute human skin irritants in this group may well represent suitable alternatives to the rabbit.

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The hazard associated with a single chemical substance or a combination of 2 or more substances (a preparation) refers to their intrinsic propensity to cause a particular effect, in this case, skin irritation. Regulatory guidelines in the European Union require that this information is provided for new substances (1), increasingly for existing substances (2) and for certain preparations (3). Traditionally, the potential of a substance or preparation to cause skin irritation/corrosion has been assessed using a rabbit skin test (4, 5). However, an alternative strategy and the associated protocol for the use of human volunteers to identify skin irritation hazard has been described (6–10). In principle, if corrosive and other toxicologically unacceptable hazards do not exist for the substance or preparation, then it is possible to conduct a human test, performed to the highest ethical standards, which is similar to the Draize rabbit skin-irritation test, but whose design limits the intensity of any skin reactions. The human 4-h patch test provides this opportunity to identify substances with significant skin irritation poten-

tial without recourse to the use of animals. The protocol is designed to avoid the production of more than mild irritant reactions and meets the highest ethical standards. The value of the method in providing gold standard data for the identification of those substances (or in principle preparations) which should, or should not, be classified as irritant to skin under European legislative rules is discussed. Special emphasis is placed also on the value of the data in the development and acceptance of potential *in vitro* alternatives to the rabbit skin test.

The Protocol

The test is described in complete detail elsewhere (9, 10), but in brief the patch test procedure involves application of 0.2 ml (0.2 g for solid test materials) on a 25 mm plain Hill Top Chamber containing a Webril pad (Hill Top Companies, Cincinnati, Ohio, USA), moistened for solid test materials, to the skin of the upper outer arm of 30 human volunteers for up to 4 h. To avoid the

production of unacceptably strong reactions, test materials are applied progressively from 15 and 30 min through 1, 2, 3 and 4 h. Each progressive application is at a new skin site. The shorter exposure periods can be omitted if the study directors are satisfied that excessive reactions will not occur following longer exposure. Treatment sites are assessed for the presence of irritation using a 4 point scale (Table 1) at 24, 48 and 72 h after patch removal. A volunteer with a + or greater reaction at any of the assessments is considered to have demonstrated a positive irritant reaction, and treatment with the causative substance does not proceed on that person. For panellists with a + or greater response at application times of less than 4 h to a particular test substance, it is assumed that they would present a stronger irritant reaction if exposed for 4 h. However, once a + or greater response is obtained, there is no need to subject these panelists to further treatment with that substance. In evaluating the results, what is measured is the number of panellists who had, or would have had, a positive irritant reaction after a 4-h exposure. The interpretation of the results in terms of EC classification is done by statistical comparison of the data with a concurrent positive control. In these studies, 20% sodium dodecyl sulfate (SDS) has always been used as this control, for reasons that have been well documented (7, 9, 10).

The Database

The results of the human 4-h patch tests conducted to date are assembled in Table 2. In total, data is presented on 65 substances, the great majority tested undiluted. For reference, particularly because specific concentration limits have been set in relation to defined EC classification levels (1), information on certain dilutions of selected substances which fall within these classifications is also given. The table includes the historic (and sometimes incorrect) EC classification (NC = not classified, R38 = irritant and

R34 = corrosive), together with how the materials should actually be classified on the basis of their acute skin irritation potential in man. The basis for the classification decisions on the prospective human data is as follows: where the incidence of positive irritation reactions to the undiluted test substance was significantly greater than or not significantly different (using Fisher's exact test) than the level of reaction in that same panel of volunteers to 20% SDS, the substance should be classified as irritant to skin (R38); where the level of reaction was substantially and significantly lower than the response to SDS, then the substance is not classified (10). It is important to note that, while certain substances appear to have been over-classified on the basis (presumably) of rabbit data, on a limited number of occasions the opposite situation applies. This reinforces the opportunity that this human test provides – that of the accurate identification of substances with significant (i.e. classifiable) acute skin irritation potential using the endpoint of concern in the species of concern.

Conclusions

In vitro alternatives for the identification of skin irritation have been the subject of investigation and development in a considerable number of laboratories for many years (11), but none of these is close to validation and acceptance by regulatory authorities. One of the difficulties is the absence of a gold standard dataset against which to validate these alternatives. For skin irritation, the approach suggested herein uses human volunteers with the acute irritant response in skin as the endpoint. Therefore, the validation issues normally associated with *in vitro* approaches do not apply (12). Although the normal validation requirements do not apply, it has been demonstrated nevertheless that the test is robust in terms of interlaboratory reproducibility (13) and that the results are applicable to all skin types and populations tested (14–17). Furthermore, it is clear that the assay provides results which are consistent between different testing laboratories (13, 18). Thus, what is proposed is that for some chemicals, if corrosive and other toxicologically unacceptable hazards do not exist, their intrinsic skin irritation potential can be defined by an ethical human volunteer study, founded on the principle of a progressive exposure regime linked to the use of a benchmark positive control substance. The validity and ethics of this approach have been reported in a relatively recent review article (19). Thus, at one and the same time, the human 4-h patch test allows avoidance of the use

Table 1. Assessment of reactions

Grading	Description of response
0	No reaction
+	Weakly positive reaction (usually characterized by mild erythema or dryness across most of the treatment site)
++	Moderately positive reaction (usually distinct erythema possibly spreading beyond the treatment site)
+++	Strongly positive reaction (strong, often spreading erythema with oedema)

Table 2. Materials tested in the human 4-h patch test (HPT)

Test Material	Existing Class ¹	HPT Class ²	Positive reactions ³	Positive to SDS control ⁴
Acetic acid (10%)	R38	NC	6/63	45/64
Alcohol ethoxylate C ₁₁ /E3	R38	NC	1/32	26/32
Alcohol ethoxylate C ₁₁ /E7	R38	NC	0/31	12/31
Alcohol ethoxylate C ₁₂₋₁₅ /E3	R38	NC	0/32	24/32
Alcohol ethoxylate C ₁₂₋₁₅ /E5 phosphate	R34	NC	1/32	23/33
Alcohol ethoxylate C ₁₆₋₁₈ /E5	R38	NC	0/27	14/27
Alcohol ethoxylate C ₁₆₋₁₈ /E14	R38	NC	0/27	14/27
Alkyl dimethyl betaine	R38	NC	3/32	12/32
Alkyl polyglucoside 600	NC	NC	1/30	28/31
Benzalkonium chloride (7.5%)	R38	R38	19/56	32/56
Benzalkonium chloride (10%)	R34	R38	4/29	8/27
Benzyl alcohol	NC	NC	1/31	17/32
Benzyl salicylate	NC	NC	0/30	20/31
Butan-1-ol	NC	NC	1/31	15/31
Butyl benzoate	NC	NC	0/30	14/30
Citronellol	R38	NC	0/30	20/31
Cocotrimethyl ammonium chloride	R38	NC	20/89	50/90
Decanoic acid	R38	R38	54/81	51/80
Decanol	R38	NC	24/159	95/159
N,N-dimethyl-N-dodecyl aminobetaine	R38	R38	30/32	27/32
Dimethylsulfoxide	NC	R38	31/31	12/31
Dodecanoic acid	R38	NC	4/61	42/62
Dodecanol	NC	NC	0/29	16/29
Ethylenediamine tetraacetic acid disodium salt	NC	NC	0/26	21/26
Ethanol	NC	NC	1/31	15/31
Eugenol	R38	NC	4/26	21/26
Geraniol	R38	NC	5/28	23/30
Heptanoic acid	R34	R38	20/31	20/31
Heptyl butyrate	NC	NC	0/30	18/31
Hexadecanoic acid	NC	NC	0/29	22/31
Hexanol	R38	NC	8/28	21/28
Hexyl salicylate	R38	NC	0/30	16/30
Hydrochloric acid (10%)	R38	NC	16/89	49/91
Hydrogenated tallow amine	R38	R38	19/19	17/19
Isopropanol	NC	NC	0/31	17/32
Isopropyl myristate	NC	NC	1/30	18/31
Isopropyl palmitate	NC	NC	0/29	17/29
Lactic acid	NC	R38	21/26	15/25
Linalyl acetate	R38	NC	1/31	12/31
Methyl caproate	NC	NC	0/29	17/29
Methyl laurate	R38	NC	0/31	15/31
Methyl palmitate	NC	NC	1/29	17/29
Octanol	R38	NC	5/28	21/28
Octanoic acid	R34	R38	48/63	38/62
n-Pentanol	NC	NC	0/30	14/30
Polyethylene glycol 400	NC	NC	0/28	12/28
Propylene glycol tertiary butyl ether	NC	NC	0/28	12/28
Potassium soap	NC	NC	0/31	9/29
C ₁₂₋₁₃ β -branched primary alcohol sulfate/1E	R38	NC	9/30	28/31
C ₁₂₋₁₃ β -branched primary alcohol sulfate	R38	R38	26/31	28/31
Propylene glycol	NC	NC	2/32	23/33
Sodium carbonate	NC	NC	0/26	21/26
Sodium dodecyl sulfate	R38	R38	54/65	43/64
Sodium hydroxide (0.5%)	R38	R38	20/33 ⁵	23/33
Sodium percarbonate	R38	NC	1/26	21/26
Sodium perborate	R38	NC	1/26	21/26
Sodium soap	NC	NC	0/31	9/29
Sodium xylene sulfonate	NC	NC	0/30	16/30
α -Terpineol	R38	NC	0/30	16/30
Tetradecanoic acid	NC	NC	0/29	22/31
Tetradecanol	NC	NC	0/29	16/29

Triethanolamine	NC	NC	0/32	26/32
Tris(hydroxymethyl) aminomethane	NC	NC	2/32	12/32
Tween 80	NC	NC	1/29	24/29
Water	NC	NC	3/59	58/59

¹NC = Not classified; R38 = Irritant to skin; R34 = Corrosive to skin; based on EC, suppliers and trade association data; SDS = sodium dodecyl sulfate.

²Classifications as above, but based on the human 4-h patch test results.

³Number of individuals with a positive irritant reaction to the test material/total panel size.

⁴Number of individuals with a positive irritant reaction to the 20% sodium dodecyl sulfate control in the same panel.

⁵Number of positive reactions after exposure only up to 1h.

of animals, provides an appropriate route to accurate classification (in EC terms) of skin irritation and generates the quality of data, the gold standard, which can then be used to validate the *in vitro* alternatives to the rabbit Draize skin test which is demanded by recent EU legislation (20). The data presented in Table 2 are offered here for that last-mentioned purpose.

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