TABLE: GRADING OF SKIN REACTIONS

Erythema and Eschar Formation

No erythema 0
Very slight erythema (barely perceptible) 1
Well defined erythema 2
Moderate to severe erythema 3
Severe erythema (beef redness) to eschar formation preventing grading of erythema 4

Maximum possible: 4

Oedema Formation

No oedema 0
Very slight oedema (barely perceptible) 1
Slight oedema (edges of area well defined by definite raising) 2
Moderate oedema (raised approximately 1 mm) 3
Severe oedema (raised more than 1 mm and extending beyond area of exposure) 4

Maximum possible: 4

Histopathological examination may be carried out to clarify equivocal responses.

ANNEX

DEFINITIONS

1. Dermal irritation is the production of reversible damage of the skin following the application of a test substance for up to 4 hours.

2. Dermal corrosion is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

SUPPLEMENT TO TEST GUIDELINE 404

A Sequential Testing Strategy for Dermal Irritation and Corrosion

GENERAL CONSIDERATIONS

1. In the interest of sound science and animal welfare, it is important to avoid the unnecessary use of animals and to minimise any testing that is likely to produce severe responses in animals. All information on a substance relevant to its potential skin corrosivity/irritancy should be evaluated prior to considering *in vivo* testing. Sufficient evidence may already exist to classify a test substance as to its dermal corrosion or irritation potential without the need to conduct testing in laboratory animals. Therefore, utilizing a weight-of-the-evidence analysis and a sequential testing strategy, will minimise the need for *in vivo* testing, especially if the substance is likely to produce severe reactions.

2. It is recommended that a weight-of-the-evidence analysis be used to evaluate existing information regarding the skin irritation and corrosion of substances to determine whether additional studies, other than *in vivo* dermal studies, should be performed to help characterise such potential. Where further studies are needed, it is recommended that the sequential testing strategy be utilised to develop the relevant experimental data. For substances which have no testing history, the sequential testing strategy should be utilised to develop the data set needed to evaluate its dermal corrosion/irritation potential. The testing strategy described in this Supplement was developed at an OECD workshop (1) and was later affirmed and expanded in the Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, in November 1998 (2).

3. Although this sequential testing strategy is not an integral part of Test Guideline 404, it expresses the recommended approach for the determination of skin irritation/corrosion characteristics. This approach represents both best practice and an ethical benchmark for *in vivo* testing for skin irritation/corrosion. The Guideline provides guidance for the conduct of the *in vivo* test and summarises the factors that should be addressed before initiating such a test. The strategy provides an approach for the evaluation of existing data on the skin irritation/corrosion properties of test substances and a tiered approach for the generation of relevant data on substances for which additional studies are needed, or for which no studies have been performed. It also recommends the performance of validated and accepted *in vitro* or *ex vivo* tests for skin corrosion/irritation under specific circumstances.

DESCRIPTION OF THE EVALUATION AND TESTING STRATEGY

4. Prior to undertaking tests as part of the sequential testing strategy (Figure), all available information should be evaluated to determine the need for *in vivo* skin testing. Although significant information might be gained from the evaluation of single parameters (e.g. extreme pH), the totality of existing information should be considered. All relevant data on the effects of the substance in question, or its analogues, should be evaluated in making a weight-of-the-evidence decision, and a rationale for the decision should be presented. Primary emphasis should be placed upon existing human and animal data on the substance, followed by the outcome of *in vitro* or *ex vivo* testing. *In vivo* studies of corrosive substances should be avoided whenever possible. The factors considered in the testing strategy include:

5. Evaluation of existing human and animal data (Step 1). Existing human data, e.g. clinical or occupational studies and case reports, and/or animal test data, e.g. from single or repeated dermal exposure

toxicity studies, should be considered first, because they provide information directly related to effects on the skin. Substances with known irritancy or corrosivity, and those with clear evidence of non-corrosivity or non-irritancy, need not be tested in *in vivo* studies.

6. Analysis of structure activity relationships (SAR) (Step 2). The results of testing of structurally related substances should be considered, if available. When sufficient human and/or animal data are available on structurally related substances or mixtures of such substances to indicate their skin corrosion/irritancy potential, it can be presumed that the test substance being evaluated will produce the same responses. In those cases, the test substance may not need to be tested. Negative data from studies of structurally related substances or mixtures of such substances do not constitute sufficient evidence of non-corrosivity/non-irritancy of a substance under the sequential testing strategy. Validated and accepted SAR approaches should be used to identify both dermal corrosion and irritation potential.

7. Physicochemical properties and chemical reactivity (Step 3). Substances exhibiting pH extremes such as ≤ 2.0 and ≥ 11.5 may have strong local effects. If extreme pH is the basis for identifying a substance as corrosive to skin, then its acid/alkali reserve (or buffering capacity) may also be taken into consideration (3)(4). If the buffering capacity suggests that a substance may not be corrosive to the skin, then further testing should be undertaken to confirm this, preferably by the use of a validated and accepted *in vitro* or *ex vivo* test (see paragraph 9).

8. Dermal toxicity (Step 4). If a chemical has proven to be highly toxic by the dermal route, an *in vivo* dermal irritation/corrosion study may not be practicable because the amount of test substance normally applied could exceed the highly toxic dose and, consequently result in the death or severe suffering of the animals. In addition, when dermal toxicity studies utilising albino rabbits have already been performed up to the limit dose level of 2000 mg/kg body weight or higher, and no dermal irritation or corrosion has been seen, additional testing for skin irritation/corrosion may not be needed. A number of considerations should be borne in mind when evaluating acute dermal toxicity in previously performed studies. For example, reported information on dermal lesions may be incomplete. Testing and observations may have been made on a species other than the rabbit, and species may differ widely in sensitivity of their responses. Also the form of test substance applied to animals may not have been suitable for assessment of skin irritation/corrosion (e.g., dilution of substances for testing dermal toxicity (5). However, in those cases in which well-designed and conducted dermal toxicity studies have been performed in rabbits, negative findings may be considered sufficient evidence that the substance is not corrosive or irritating.

9. Results from *in vitro* or *ex vivo* tests (Steps 5 and 6). Substances that have demonstrated corrosive or severe irritant properties in a validated and accepted *in vitro* or *ex vivo* test (6)(7) designed for the assessment of these specific effects, need not be tested in animals. It can be presumed that such substances will produce similar severe effects *in vivo*.

10. *In vivo* test in rabbits (Steps 7 and 8). Should a weight-of the-evidence decision be made to conduct *in vivo* testing, it should begin with an initial test using one animal. If the results of this test indicate the substance to be corrosive to the skin, further testing should not be performed. If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals for an exposure period of four hours. If an irritant effect is observed in the initial test, the confirmatory test may be conducted in a sequential manner, or by exposing the two additional animals simultaneously.

LITERATURE

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(2) OECD (1998). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, November 1998 (http://www1.oecd.org/ehs/Class/HCL6.htm).

(3) Worth, A.P., Fentem J.H., Balls M., Botham P.A., Curren R.D., Earl L.K., Esdail D.J., Liebsch M. (1998). An Evaluation of the Proposed OECD Testing Strategy for Skin Corrosion. ATLA 26, 709-720.

(4) Young, J.R., How, M.J., Walker, A.P., Worth, W.M.H. (1988). Classification as Corrosive or Irritant to Skin of Preparations Containing Acidic or Alkaline Substances, Without Testing on Animals. Toxic In Vitro, 2 (1) pp 19-26.

(5) Patil, S.M., Patrick, E., Maibach, H.I. (1996) Animal, Human, and In Vitro Test Methods for Predicting Skin Irritation, in: Francis N. Marzulli and Howard I. Maibach (editors): Dermatotoxicology. Fifth Edition ISBN 1-56032-356-6, Chapter 31, 411-436.

(6) Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Edsail, D.J., Holzhutter, H.G. and Liebsch, M. (1998). The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the Management Team. Toxicology in Vitro 12, pp.483 – 524.

(7) EU (2000). Official Journal of The European Communities L136/91 of 8 June 2000, Method B.40 Skin Corrosion.

FIGURE

TESTING AND EVALUATION STRATEGY FOR DERMAL IRRITATION /CORROSION

	Activity	Finding	Conclusion
1	Existing human and/or animal data showing effects on skin or mucous membranes	Corrosive	Apical endpoint; considered corrosive. No testing is needed.
		Irritating	Apical endpoint; considered to be an irritant. No testing is needed.
		Not corrosive/not irritating	Apical endpoint; considered not corrosive or irritating. No testing is needed.
	↓ *No information available, or available information is not conclusive*		
2	Perform SAR evaluation for skin corrosion/irritation	Predict severe damage to skin	Considered corrosive. No testing is needed.
		Predict irritation to skin	Considered an irritant. No testing is needed.
	↓ *No predictions can be made, or predictions are not conclusive or negative*		
3	Measure pH (consider buffering capacity, if relevant)	pH ≤ 2 or ≥ 11.5 (with high buffering capacity, if relevant)	Assume corrosivity. No testing is needed.
	↓ *2<pH < 11.5, or pH ≤2.0 or ≥ 11.5 with low/no buffering capacity, if relevant*		
4	Evaluate systemic toxicity data via dermal route [1]	Highly toxic	No further testing is needed.
		Not corrosive or irritating when tested to limit dose of 2000 mg/kg body weight or higher, using rabbits	Assume not corrosive or irritating. No further testing is needed.
	↓		

[1] Can be considered before Steps 2 and 3.

Such information is not available or is non-conclusive

↓

5	Perform validated and accepted *in vitro* or *ex vivo* test for skin corrosion	Corrosive response	Assume corrosivity *in vivo*. No further testing is needed.

↓

Substance is not corrosive, or internationally validated in vitro/ ex vivo testing methods for skin corrosion are not yet available

↓

6	Perform validated and accepted *in vitro* or *ex vivo* test for skin irritation	Irritant response	Assume irritancy *in vivo*. No further testing is needed.

↓

Substance is not an irritant, or internationally validated in vitro or ex vivo *testing methods for skin irritation are not yet available*

↓

7	Perform initial *in vivo* rabbit test using one animal	Severe damage to skin	Considered corrosive. No further testing is needed.

↓

No severe damage

↓

8	Perform confirmatory test using one or two additional animals	Corrosive or irritating	Considered corrosive or irritating. No further testing is needed
		Not corrosive or irritating	Considered not corrosive or irritating. No further testing is needed