J. Soc. Cosmet. Chem. **26**, 173–187 (1975)

Exaggerated exposure in topical irritancy and sensitization testing

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Presented on 26–30th August 1974 in London at the IFSCC VIIIth International Congress on 'Cosmetics—Quality and Safety' organized by the Society of Cosmetic Chemists of Great Britain.

Synopsis—The concept of a 'safety margin' provides a convenient expression for the hazard of adverse reaction following topical administration. Exaggeration of exposure conditions in PREDICTIVE TESTING helps to establish the safety margin, but reliability of any prediction depends on limiting the effects of exaggeration to quantitative rather than qualitative enhancement of responses. Gross exaggeration often leads to qualitative changes defying interpretation in terms of hazard during normal use.

Techniques for safety evaluation based upon causing only threshold effects and comparison of an unknown with a well-established 'control' preparation of similar type are suggested as most suitable for relatively innocuous cosmetics. Human tolerance tests would probably be ideal for the purpose but extremely time-consuming. If animal tests are used to screen for skin and EYE IRRITANCY, there should not be any need for grossly exaggerated exposure since the species mostly used approximate quite closely to man in their susceptibility to skin and eye irritants.

The prediction of sensitizing potential by exaggerating exposure is unsatisfactory owing to insufficiency of data on DOSE-RESPONSE behaviour for mild SENSITIZERS. Experience in normal use of a cosmetic by gradually increasing numbers of individuals would seem to be the only available way to establish SENSITIZING POTENTIAL for cosmetic formulations, although a GUINEA-PIG TEST may be useful for screening new raw materials.

INTRODUCTION

The possible hazard of adverse reaction in response to topical administration needs to be assessed by means of suitable predictive tests. The extent of such a hazard is conveniently expressed in terms of the margin

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between probable exposure of the skin or mucous membranes during normal use and the level of exposure which would produce an adverse reaction. In order to err on the side of safety, broad margins of safety are sought by exaggerating the levels and conditions of exposure in the test procedures. However, injury may result from grossly excessive direct contact of many tissues with even the most innocuous environmental chemicals and so a rational approach to exaggeration is essential.

The degree of chemical insult that the skin or mucous membranes might be expected to tolerate is somewhat problematical. The extensive testing carried out on the safety of ingested materials, such as food additives, offers little guidance as such investigations are principally aimed at demonstrating toxic effects after systemic absorption. The aspect of safety evaluation for ingested substances corresponding to topical administration is the direct effect, if any, of the test materials on the gastro-intestinal lining. Severe irritation of the lining would indeed be observed under conditions of gross exposure to many universally-accepted food materials and especially condiments such as vinegar and mustard. In other words, although there should be a wide margin of tolerance once a test material has been diluted in the body fluids following absorption, a narrower margin is to be expected in the case of a tissue in direct contact with the test material.

Whilst acknowledging that there are likely to be considerable differences between direct exposure and exposure after absorption, it might be instructive to consider the postulates on which the safety evaluation of food additives is based. A hundredfold safety factor (1) is commonly quoted; this may be deemed to offer a tenfold allowance for the greater susceptibility to systemic toxicants of man compared to laboratory animals, together with a further tenfold allowance for variation in susceptibility between individuals. In terms of systemic toxicity, such a tenfold allowance for inter-species differences in metabolic transformation and excretion seems reasonable. Effects on the skin and mucous membranes, however, are not primarily dependent on species-specific metabolic pathways. Indeed, the skin of those mammals most often used for irritancy testing approximates quite closely to human skin in its susceptibility to irritation or even shows greater sensitivity to some irritants (2, 3).

The epidermal horny layer of the skin is an important barrier to the absorption of foreign chemicals (4) and provides the first line of defence against irritants. Thickness of the horny layer varies across the human skin and in some regions it is thinner than the horny layer of other mammals. However, human epidermis overall is much thicker (5) than in most

mammals, including the rabbit, rat and mouse; this probably explains why applied substances do not easily penetrate human skin (6) and why, on the whole, it is no more susceptible to irritants than is the skin of these species. When irritancy tests are carried out on animal skin, it would therefore be irrational to allow a tenfold margin for interspecies differences.

The possible irritancy of cosmetic materials in contact with tissues of the eye is usually studied by instillation into the conjunctival sac of the rabbit eye. On the basis of wide experience of such tests, Davies (7) suggested that the rabbit eye was decidedly more sensitive to irritants than the human eye. Thus no allowance for interspecies differences seems necessary for extrapolating rabbit eye test results in terms of hazard to man.

A major factor involved in selecting the appropriate levels of exaggeration in irritancy testing is that a quantitative enhancement of responses may help to establish a meaningful safety margin, whereas a qualitative change in the type of response could well render the findings incapable of interpretation; qualitatively atypical responses might well result from gross exaggeration of exposure levels in tests for skin and eye irritancy (*Fig. 1*). To ensure that testing procedures give the information required for safety assessment, a critical re-appraisal of current methods is needed.

Test methods

In the study of systemic toxicity, suitably exaggerated dose-levels are administered to laboratory animals in the diet, by gavage or by injection. Exposure of the skin or mucous membrane to substantially exaggerated quantities of test material is seldom practical in the study of irritancy. Direct contact within a circumscribed area is essential; an exaggerated quantity applied to a larger area will not necessarily intensify the response.

Exposure to a raw material may often be exaggerated by applying concentrated solutions, but this would not be feasible for complete formulations. Even with raw materials, unrealistic effects may occur, for example, owing to hypertonicity or grossly abnormal hydrogen ion concentration; such exaggeration could well produce effects totally irrelevant to the hazards of ordinary use, by producing qualitative rather than quantitative differences in response.

An alternative way of exaggerating topical exposure is to lengthen the time of contact compared with normal use or to make multiple applications. This is helpful if it influences the response quantitatively without provoking

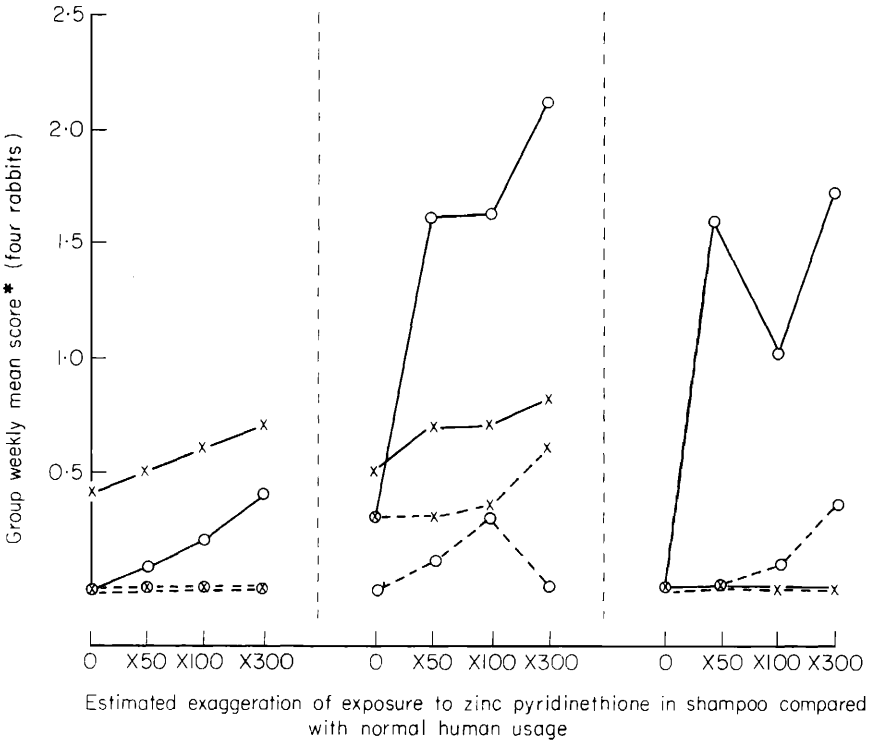

Figure 1. Comparison of skin irritancy produced by repeated shampooing (broken lines) and occlusive patch testing (solid lines). Breakage of the skin under occlusion suggests a qualitative change indicating excessive exaggeration. X, Erythema; O, breakage. *Scored according to Draize, J. H. (1959). Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.

a qualitatively different skin reaction. An example of a qualitatively altered response sometimes occurs when multiple exposures to a moderate irritant lead to an enhanced 'fatigue' response (8); this would be irrelevant in the study of short-term hazards. Thus fatigue may be pertinent to the safety of a face cream for daily use but not to a hair-waving lotion used only two or three times per year. Exaggeration by means of multiple applications should therefore be reserved for testing substances intended for frequently repeated topical use.

Another method of exaggerating exposure for irritancy testing is to apply the test material to damaged skin, which is more readily penetrated by irritants than intact skin (9). Damage may be artificially induced by abrasion, adhesive tape stripping or chemical pre-treatment. Direct effects

due to contact with the underlying tissues, however, may prove misleading if extrapolated in terms of normal skin with an intact horny layer. Certainly it is helpful to know what will happen when a product is applied to damaged skin, but simple quantitative relationships to irritancy for normal skin cannot thus be established and the distinction needs to be recognized.

Chemical pre-treatment of the skin, for example, by applying formaldehyde or sodium lauryl sulphate, may not produce grossly visible damage but will in many cases enhance penetration. Usually the degree of enhancement cannot be quantified in terms of relative irritancy to normal skin and the predictive value of a provocative test using chemical pre-treatment is therefore questionable. Furthermore, the intensity of adverse effects may be too severe to regard as reasonably justifiable for either animal or human studies.

Since the various methods used for exaggerating exposure by inflicting damage to the skin in one form or another so often produce difficulty in interpretation, a rational conclusion is that such damage should never exceed the minimum necessary to ensure a detectable response; discrimination between test materials in terms of skin irritancy may even be improved by limiting the overall response, e.g. by testing after dilution of the product (*Table I*).

Techniques involving grossly exaggerated exposure have led to serious problems of interpretation not only in skin irritancy testing but also in studies of eye irritancy. For example, it has long been customary to instil a

Table I. Improved discrimination between irritancy of shampoos applied to rabbit skin at 10% dilution

	Irritancy* after				
	5 h		24 h		
Type of shampoo	Neat	10%	Neat	10%	
Baby—based on amphoteric detergents	7	1.1	4	0	Erythema
	1.5	1	2	0	Oedema
Normal—based on anionic detergents	7	1.5	15	1.5	Erythema
	8.5	1	14	0.5	Oedema
Medicated—based on 0.5% Zn pyridinethione and anionic detergents	10.5	6	17	7.5	Erythema
	6	1	15.5	7	Oedema

* Scores according to Draize (Group means for six rabbits).

fixed quantity of undiluted test material into the conjunctival sac of the rabbit eye without, as well as with, a subsequent rinse (10). The effects produced by instillation of undiluted material without rinsing may be qualitatively, as well as quantitatively, different from the results likely to occur during normal use of the material. Consequently, products are rarely if ever deemed unfit for human use because of severe eye irritation when tested under these grossly exaggerated conditions in rabbits. A safety evaluation test procedure giving rise to severe effects which are commonly and quite properly ignored, obviously has little predictive value and no justification in terms of the suffering caused to the experimental animals. A more meaningful way of designing studies concerned with eye irritancy, as well as skin irritancy, is to employ test conditions resulting in threshold or minimal irritation and to include a material with known irritancy in the study to serve as a control. Whenever possible, the control material should be closely similar in chemical structure and mode of use to the test material.

Gaunt and Harper (11) reported a procedure whereby shampoo diluted to 10% concentration was instilled into the rabbit eye with no subsequent rinse. This technique avoids grossly unrealistic exposure but it would still be expected to give some enhancement of irritant effects by eliminating the rinsing procedure. As the authors acknowledged and we have confirmed, their technique has the apparent disadvantage of militating against the recognition of any tendency to corneal or iridial injury (*Table II*), but it may nevertheless have better predictive value than a test in which shampoo is instilled at 100% concentration. Improvements might be made by varying the shampoo concentration to some extent or by giving duplicate instillations (12).

Table II. Rabbit eye test findings showing effect of dilution of shampoos with water. Results at 10% dilution apparently gave better prediction of human response, no corneal or iridial injury having been notified as consumer complaints

	100% shampoo (no rinse)			10% shampoo (no rinse)		
	Cornea	Iris	Conjunctiva	Cornea	Iris	Conjunctiva
Non-medicated liquid shampoo	0	0	68	0	0	4
Non-medicated cream shampoo	35	10	108	0	0	12
Medicated cream shampoo	25	10	90	0	0	62

Scores according to Draize; each figure gives sum of scores for three rabbits after 1, 2, 3, 4 and 7 days.

The questionable features of the more usual form of rabbit eye test, apply particularly to its use in the evaluation of detergent ingredients and shampoo formulations. For cosmetic products, however, normally used away from a washbasin and without rinsing, the instillation of undiluted product may be more meaningful and seldom leads to gross injury in the rabbit eye.

A particular difficulty in using laboratory animals is the choice of species and testing site for products such as dentifrices coming in contact with mucous membranes during normal use. The hamster cheek pouch has sometimes been used for predictive testing although the presence of a cornified epithelial lining of the buccal mucosa in this species may limit the sensitivity of the test. In view of the ease of handling of these animals and the relatively large area of tissue available for examination and biopsy, this seems nevertheless to be the test of choice at the present time; possible lack of sensitivity may be overcome by reasonable exaggeration of product concentration and duration of contact with the cheek pouch.

The need to limit irritant effects in the course of tests on human volunteers is obvious, to avoid causing harm and to ensure continuing availability of willing panellists. It is also highly desirable to maintain and, if possible, to improve standards in the humanitarian treatment of laboratory animals and close attention should be given to the design of suitable procedures both for human and animal testing. The tests should preferably not, however, be designed in such a way that they mostly lead to purely negative findings, since these are as hard to interpret as grossly abnormal positive results; this is an additional reason for favouring a threshold irritancy approach.

Designs for skin irritancy studies based on threshold irritation have been put forward by Kligman and Wooding (13). These authors suggested that findings should be recorded in terms of ID50 (*concentration* to yield threshold irritant effect in 50% of test subjects) or IT50 (*time* of exposure for threshold irritation in 50% of subjects). Choice between the two methods of expressing results would depend on the feasibility of testing diluted product and the level of irritancy of the test material. Whereas the ID50 or IT50 principle may be suitable for evaluating new raw materials, formulated cosmetics will often prove altogether too bland for such an approach and are more readily tested by direct comparison with an appropriately-chosen control product.

PATCH TESTING

Skin irritancy is usually investigated by means of a patch test procedure (14); frequently this involves the application of test material to the skin with relatively prolonged occlusion under an impermeable or semi-permeable dressing (*Table III*). Occlusion itself will produce minimal damage of the skin and a 'closed' patch test therefore embodies some degree of exaggerated exposure.

Table III. Occlusivity of patch test materials expressed as drying time of hydrated $CoCl_2$ paper on glass backing, exposed at $19.5 \pm 1°$, $49.5 \pm 1.5\%$ RH, beneath the patches, for pink → blue colour change

Material	Mean drying time (min)	n=	SD
Nil	20	7	6.3
Gauze (only)	45	6	7.8
Gauze + blenderm-backed lint square	65	6	8.4
Micropore + lint square	80	6	6.3
Micropore (only)	155	7	6.3
Micropore + blenderm-backed lint square	200	7	33.0
Dermicel + blenderm-backed lint square	230	6	21.0
Dermicel (only)	390	7	8.4
Blenderm (only)	1145	7	43.0

Micropore: supplied by 3M Co, London.
Dermicel: supplied by Johnson & Johnson Ltd, Slough.
Blenderm: supplied by 3M Co, London.

In the hands of an experienced clinician, the occlusive patch test is a valuable technique for diagnosing the causal agents of pre-existing irritation and sensitization. Using occlusive testing in a prophetic manner, however, involves different considerations. Some preparations (antiperspirants, for example) are normally used under conditions tantamount to occlusion; relevance of an occlusive patch test is then obvious. For many other products, e.g. face creams and shampoos, occlusive patch tests may be somewhat less realistic. However, an occlusive or partially occlusive prophetic patch test is probably the best available procedure for predicting the effects of long-term exposure by means of a relatively short-duration test, taking into account the fact that many toiletries and cosmetics are used repeatedly over long periods of time (*Tables IV and V*). Data showing quantitative results for occlusive *vs* non-occlusive exposure in 21-day human patch tests (2) indicate that occlusion gives a substantial enhancement of effect

for many irritants. Skin irritancy testing carried out under 'open' patch test conditions (e.g. with the applied material under a loosely-woven gauze covering) might therefore be preferable in order to avoid too many 'false positive' results. However, since toiletries and cosmetics are invariably formulated to give minimal skin reaction, 'open' patch testing in practice would nearly always yield wholly negative results which would be hard to interpret. 'Closed' patch tests resulting in threshold irritation, preferably including controls with known irritant potential, allow decisions on the acceptability of a cosmetic ingredient or product to be reached with greater confidence.

Another important consideration in patch testing concerns the form and amount of test material applied. When a volatile solvent is present in the formulation, this should obviously be permitted to evaporate before a 'closed' patch is applied to the skin; if this precaution is not taken, irritant effects due to the solvent will tend to give 'false positive' responses in the sense that solvent evaporation during normal use would avoid any irritation from this source. A further complication is that some toiletry products are

Table IV. Detection of moderate increases in skin irritation, using partially occlusive human patch test (Uttley, M. and Van Abbé, N. J. *J. Soc. Cosmet. Chem.* **24**, 217 (1973))

Product	Irritancy score
Nil (blank lint patch)	6.6*
Lotion base	6.6
Base + DHA (aged)	8.4
Base + DHA (aged) + fresh DHA**	9.3
Base (fresh) + fresh DHA (10%)	10.9*

DHA = Dihydroxyacetone.
* Difference significant at 1% level (Wilcoxon).
** Adjusted to 10% concentration.

Table V. Correlation between partially-occlusive human patch test (Uttley and Van Abbé) and consumer reports

	Irritancy score	Interpretation of score	Consumer reports
Moisturizing cream	6.3*	Non-irritant	No irritation
Cheek gloss no. 1	11.0+	Slight irritant	No irritation
Cheek gloss no. 2	12.6*	Slight irritant	No irritation
Cheek gloss no. 3	18.1	Moderate → severe irritant	Irritation

* Significant at < 1% level (Wilcoxon).
+ Significant at < 2% level (Wilcoxon).

rinsed off the skin during normal use soon after application. In these instances, loading a patch with the undiluted product and leaving it in contact with the skin at full concentration for many hours is highly unrealistic and may well prove intolerable for the volunteers.

For a shampoo, a more informative method of patch testing is to apply the product to the patches at a dilution of 10–20% with water. When a physiologically-active constituent occurs in the formula (e.g. an antimicrobial agent) an alternative procedure is to load the patch with a quantity based on an amount per unit area of skin equivalent to the residue left on the scalp after shampooing, as determined by assay.

Where the assay of actual residues presents serious difficulty, open patch tests may be carried out by actually shampooing a small test area of skin daily for several days; experience suggests that this procedure may even have better predictive value than a closed patch test based on estimates of residual quantities after rinsing.

There should thus be no insuperable difficulty in showing an adequate safety margin for the topical administration of cosmetics and toiletries by reasonable exaggeration of the exposure conditions in tests for irritancy. Since, for the reasons already stated, the present authors contend that no allowance is usually necessary for interspecies variation, the choice between using human volunteers or laboratory animals does not appear to have great significance from the investigator's standpoint. If, however, the irritant potential of the test material really is unknown, initial screening is certainly best carried out with laboratory animals. Moreover, if effects on damaged skin are being examined, an animal screening test is obviously desirable before extending the study to human skin, as a reasonable safeguard for the volunteers. Despite the similarity in responses to irritants generally shown, if interspecies differences are discernible when results for animal and human irritancy tests are compared, greater reliance should obviously be placed on the human data.

To avoid uncertainty in extrapolation from animal responses to man whilst minimizing the risk of serious harm to volunteers, human studies may sometimes take the form of tolerance tests. The degree of exposure (with respect to amount of test material applied, its concentration or the duration of contact) is gradually increased until a threshold response occurs. The predictive value of such a test should be satisfactory provided that precautions are taken to avoid fatigue by careful choice of site of application or interval between applications. Human eye irritancy testing should generally follow this type of cautious approach (12). The drawback to more

widespread use of the human tolerance test, however, is its time-consuming nature and the difficulty of recruiting volunteers.

SENSITIZING POTENTIAL

Sensitization is an important type of possible adverse reaction to toiletries and cosmetics; it usually involves the risk of causing an allergic contact dermatitis and needs to be considered separately from irritancy. Allergens may sensitize occasional individuals at concentrations which are without any significant effect on the majority of the population; this feature of allergenicity causes great difficulty in predictive testing. Some dermatologists nevertheless take the view that exposing a group of randomly-chosen subjects under test conditions to an exaggerated concentration of a suspected allergen increases the chance of recognizing its sensitizing potential. If so, what degree of exaggeration is appropriate?

Marzulli and Maibach (15) recently reported a detailed investigation of sensitizing properties using exaggerated concentrations of test materials. For example, with sorbic acid tested at 20% concentration, one of the 33 subjects they tested gave a positive reaction. The crucial question is what proportion of users, if any, would be sensitized at a typical use-concentration of about 0.1–0.2%. Clearly this is unanswerable without knowing the shape of the dose-response curve for an allergen of the same or a similar type, extending right down to normal use-level concentrations of the test substance. These authors did, in fact, conduct tests at several concentration levels with a number of compounds but the proportion of subjects with positive responses did not show a dose-response pattern justifying any broad conclusions; a graded dose-response relationship may perhaps be inferred on theoretical grounds but even the extensive work carried out by Marzulli and Maibach (15) was evidently insufficient to demonstrate it clearly.

Some toxicologists concerned with toiletries and cosmetics prefer to conduct tests for sensitizing potential at normal use-concentration of the test materials, using human volunteers. If such tests involve multiple exposures under occlusive patches, the resulting minor degree of skin damage should marginally increase the likelihood of a positive response. However, since it is well known that toiletries and cosmetics in general sensitize less than, say, 1 in 10 000 users, the predictive value of any use-concentration test carried out with only a few hundred volunteers must be exceedingly

small. Selection of atopic subjects for test panels is sometimes considered to improve predictive value but the evidence to indicate that atopics show enhanced susceptibility to topical allergens in general is questionable (16).

Bearing in mind the objections to grossly exaggerated exposure testing and recognizing that sensitization testing at normal use-concentration often yields negative results that cannot be interpreted or which may be unreliable, there are certain attractions in devising a test procedure to enhance responses to use-concentrations and to ensure that positive results are obtained even with moderate or mild sensitizers. Kligman (17) in proposing his 'maximization' test, offered a procedure giving 24/24 positive responses to *p*-phenylenediamine even though he was still unable to detect some known mild sensitizers such as lanolin. The value of this type of test for cosmetic evaluation does not therefore yet seem to have been established.

At the present time, despite the energetic attempts by a number of highly-skilled investigators, there is clearly no satisfactory way of predicting the sensitizing potential of cosmetics and toiletries by means of human volunteer studies; nevertheless, such studies carry a definite risk of sensitizing volunteers, possibly for some years (18), and their justification is therefore doubtful. As an alternative, a reliable test for sensitizing potential using laboratory animals would obviously be helpful.

Whereas the response of some other mammals closely resembles the human response to irritants, there are marked interspecies differences in allergic responses. Suitability of the guinea-pig for sensitization testing has been validated to some extent (19) but it would be unwise to expect guinea-pig studies to eliminate any but the most potent sensitizers. Thus, although it is reasonably practical to test for the sensitizing potential of raw materials by conducting challenge tests at elevated concentrations using animals or man, no comparable procedure is yet available for studying complete formulations likely to display no more than mild sensitizing ability. Rather than applying maximizing procedures of dubious predictive value, it is probably better to allow a product to be used normally by gradually increasing numbers of individuals. This view takes for granted a prior scrutiny of the raw materials to eliminate any potent known sensitizers and an adequate scheme for monitoring adverse reactions if they are reported by users of the product.

Conclusion

Unreasonable criteria for assessing the potential hazards of topical administration do not necessarily help to protect the consumer. Thus, although animal feeding tests on a proposed food colour may well show that the maximum no-untoward-effect level is several hundred times greater than the expected human intake, even the most harmless materials applied to the skin with such exaggeration are likely to prove injurious. Frazer (1) claimed that the acceptable usage level of a substance—he was referring specifically to food additives, though others have applied his concept more widely—should be regarded as one-hundredth of the level required to produce significant modification of structure or function in not more than 50% of a group of test animals. Further, at a dose-level equal to one-tenth of the ED50, no significant changes of any kind should occur. Such margins, however, could not be applied generally to substances coming in contact with the skin or mucous membranes. For example, none of the synthetic anionic surfactants would be acceptable if a shampoo had to be formulated with no more than one-hundredth of the detergent concentration giving threshold irritation in a closed patch test. Nevertheless, present-day shampoos are used almost universally with minimal known adverse effect; a different basis for judging acceptability is therefore needed and one of the possible approaches might be to seek a tenfold margin in relation to experimental findings to allow for individual differences in susceptibility. As already shown, no allowance for interspecies differences need usually be made in irritancy testing. A tenfold safety criterion on these lines may prove quite helpful for the safety evaluation of raw materials but it will seldom be a technically feasible criterion for use in testing formulated products. Direct comparison of a newly-formulated product in a threshold irritancy test with other formulations of similar type, whose effects during normal use are known, will be more appropriate. Such a comparison will certainly give practical guidance on probable safety-in-use. A study on human volunteers will clearly be the most reliable and ideally the study should take the form of a comparison with a known 'safe' and a known 'unsafe' material of similar type (i.e. with 'negative' and 'positive' controls). Comparison with a 'positive' control (e.g. a known irritant) might facilitate quantitative expression of the findings, if a human tolerance test is carried out. In circumstances where laboratory animal studies are judged to be required, it will be equally desirable to conduct these as threshold irritancy tests to forecast the onset of hazard to man in normal use.

Reasonably exaggerated exposure in cosmetic safety evaluation may theoretically be achieved by designing 'in use' tests. Human volunteers use the test material for a few weeks with more frequent applications than would normally be made and subject to repeated examination for adverse effects. Such a procedure may involve a risk that the investigator will be unable to control the amount and frequency of application effectively and that comparisons with suitable controls may be difficult to arrange. Unless the conditions of testing prove suitable for achieving threshold responses, interpretation may depend on negative findings which will limit the reliability of the study. 'In use' testing warrants serious consideration, however, as an alternative to the highly empirical, grossly exaggerated procedures currently favoured by some investigators.

ACKNOWLEDGMENT

The authors are indebted to Mr J. Brown for the data given in *Table III.*

(*Received: 10th April 1974*)

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