A comparison between expert clinical assessment of erythema and instrumental measurements

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Introduction

The prediction of human cutaneous irritation has moved away from being primarily based on the use of experimental animals. The most widespread method applied was that based on the original procedures of Draize et al involving the rabbit, but some workers have employed other species such as mice, guinea pigs, or domestic pigs. There are however, inherent problems of extrapolating from animals to humans. There are also practical, economic and ethical reasons for attempting to devise alternatives to Draize type tests. Whilst some progress has been made in terms of alternative in vitro test systems, in vivo methods using human volunteers are more easily interpretable and are able to predict clinically relevant consumer end points, such as erythema, oedema, scaling and other undesirable consequences of exposure to an irritant. In vitro acute toxicology models, using exposures up to 48 hours to test compounds or cosmetic formulations are able to provide some data on likely irritation potential but cannot describe the range of consumer relevant end points described above that can manifest despite low toxicology irritation ranking. We have previously published work examining the optimum methodology for cutaneous irritation testing, including the influence of exposure time and occlusive chamber size in predicting irritation in even very weakly irritant cosmetic products. In this paper, we present research using experimental patch test models with surfactants and blends used in personal care products. In these studies, we sought to determine the correlation between visual scoring of erythema by trained expert assessors, with measurement of erythema using a Chromameter™ and skin temperature using an infra-red non-contact thermometer. In addition, we sought to correlate the degree of irritation with histological changes observed using invivo confocal microscopy of the skin.

Methods

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1.1 Design of study

The studies reported here were all undertaken with the protocol described below, with additional instrumentation, as documented on certain studies. The studies were blind evaluations of test products and controls in panels of 25 volunteers. Each volunteer received all products to the designated test sites on the back for five days.

The applications were continuous and under occlusion with inspection and assessment of the test site 20 minutes (+ 10 minutes) post patch removal. Sites were assessed for irritant reactions using 0-6 ranking scales for erythema and descriptive clinical terms.

1.2 Randomisation and blinding procedures

The studies were blind. The application of the test products to each test site was randomised according to a pre-prepared randomisation code.

1.3 Details of subjects

Twenty-five (25) non-patient volunteers, male or female, age range 18 – 70 years, were randomly recruited by telephone, social media and word of mouth from the test panel of Cutest for each study.

1.4 Inclusion criteria

1. Volunteers who are in the age range 18- 70 years.
2. Volunteers with no significant concurrent illnesses or skin disease.
3. Volunteers who have signed the consent form after the nature of the study has been fully explained.

1.5 Exclusion criteria

1. Pregnant or breast feeding or lactating females.
2. Volunteers who take any systemic or topical medication likely to interfere with the study e.g. anti-inflammatory drugs such as systemic steroids.
3. Volunteers who have taken part in a Health Research Authority or MHRA regulated clinical trial (e.g. at a hospital or phase I unit) within the previous eight weeks.
4. Volunteers who have taken part in a study involving the test site during the previous four weeks.
5. Volunteers with a recent history (previous 12 months) of significant skin disease requiring medical intervention, e.g. Dermatology outpatient appointment.
6. Volunteers with an allergy likely to interfere with the study.
7. Volunteers whose skin has been excessively exposed to the sun or to UV rays during the previous two weeks.
8. Volunteers with an illness, or who are currently taking medication, which results in impaired wound healing.

1.6 Medical history

Each subject participating in the study had a health review and skin examination before joining the test panel of Cutest. In addition, a study nurse updated each subject's medical history immediately prior to participation in this study.

2. MATERIALS

2.1 Sample preparation

Surfactant solutions used in the studies were those typically used in personal care and were diluted to 0.2% w/v in distilled water on each day of application.

The test surfactants used were as follows:

Sodium lauryl sulphate (SLS)

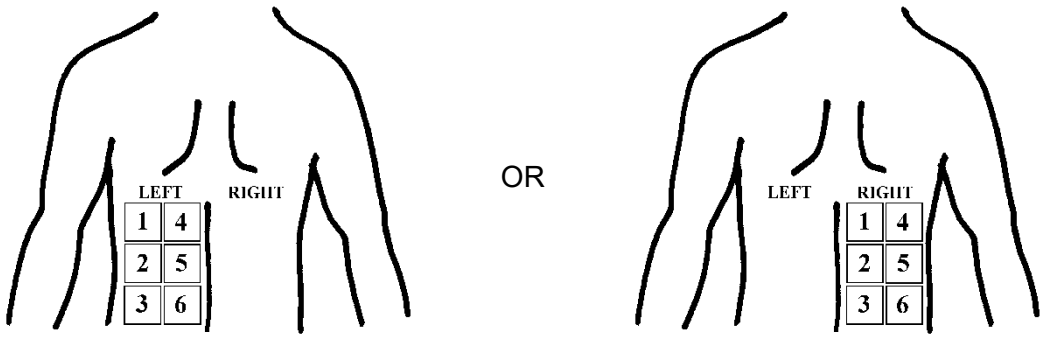
Sodium lauryl ether sulphate (SLES)

Cocamidopropyl betaine (CAPB)

blends of CAPB/SLES

2.2 Test sites

The test site for the irritancy studies were the mid to lower part of the back between the waistline and the mid-point between the waist and the shoulders, avoiding the area over the vertebral column. The test materials were applied to either the left or the right side of the mid to lower back as shown in the figure below:



2.3 Application schedule

The products were in continuous contact with the skin of the mid to lower back over a five-day period. The test patches were removed, the sites assessed, and new identical test patches applied to the same site using the following schedule:

Day 1	Mon	Baseline measurements. Apply products under occlusion	
Day 2	Tues	Remove, wait a minimum of 20 min. assess sites, measure	Re-apply
Day 3	Wed	Remove, wait a minimum 20 min. assess sites, measure	Re-apply
Day 4	Thurs	Remove, wait a minimum 20 min. assess sites, measure	Re-apply
Day 5	Fri	Remove, wait a minimum 20 min. assess sites, measure. End of Study.	Not re-applied

2.4 Test chamber removal and site assessment

The test patches were removed carefully, and the back wiped with a gauze swab to remove any remaining test products. The sites were assessed after a minimum of 20 minutes (+ 10 minutes) to allow any reactions due to the physical removal of the adhesive tape to subside.

2.5 Erythema

At each assessment time the sites were graded for erythema on a published[1] 0-6 ranking scale as follows:

0 = No reaction.
0.5 = Slight, patchy erythema.
1 = Slight uniform erythema.
2 = Moderate, uniform erythema.
3 = Strong erythema.
4 = Strong erythema, spreading outside patch.
5 = Strong erythema, spreading outside patch with either swelling or vesiculation.
6 = Severe reaction with erosion

2.6 Clinical signs

If in addition to erythema other clinical signs of cutaneous irritation were present the following letters will be appended to the numerical score in the case report form:

OE = Oedema
V = Vesiculation
S = Scaling
C = Cracking or crazing
SC = Scabbing
P = Papules
SO = Reaction spreading outside test area
G = Glazing
N = None

2.7 Chromameter Measurements

Erythema was measured using a Chromameter CR400 (www.konicaminolta.com). The Chromameter is a tristimulus colour analyser that measures the reflected colour according to the CIE 1976 L*a*b* (CIELAB) colour space values. In this system, a* corresponds to the red green axis of a colour and is taken as a measure of erythema. The parameter a* was recorded following three repeated measurements at each test site. Three baseline measurements were taken at three locations within the test area on Day 1 prior to patch application. At subsequent visits (Day 2, 3, 4 and 5), measurements were taken at all test sites once the visual assessments had been completed.

[1] Dykes P J & Marks R (1992). An evaluation of the irritancy potential of povidone iodine solutions: Comparison of subjective and objective assessment techniques. *Clinical & Experimental Dermatology* 17, 246-249.

2.8 Infra-red thermometry Measurements

Skin temperature was measured with a non-contact infra-red thermometer (RayTemp™4) Three baseline measurements were taken on Day 1 prior to patch application. This was taken at a chosen site within the test area. At subsequent visits (Day 2, 3, 4 and 5), measurements were taken at all test sites once the visual assessments had been completed.

3. DATA EVALUATION

3.1 Irritancy

No statistical evaluation is normally carried out for this type of study. The irritancy potential is determined by the number of subjects reacting during the study and the severity of those reactions.

3.2 Cumulative irritancy

The erythema scores will be categorised into the number of volunteers with each grade of reaction at each time point.

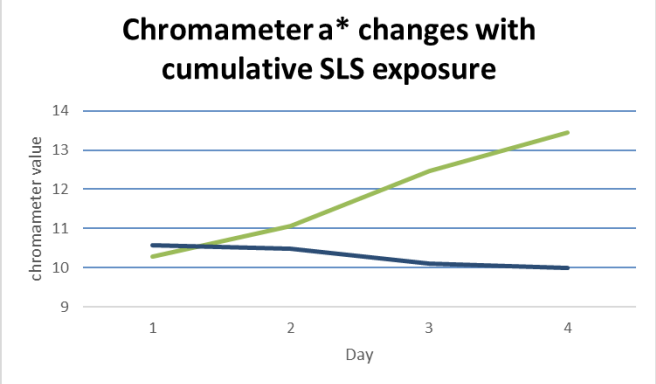
In addition, the cumulative irritancy scores will be determined by summing the erythema score at each test site in each individual over the study period, i.e. the score for each site at Days 2, 3, 4 and 5 will be summed to give the cumulative score for that site in that individual. Summary statistics (mean, standard deviation, median, minimum, maximum) will be prepared for the cumulative irritancy scores.

The cumulative irritancy scores will be compared statistically using the non-parametric Friedman Two Way ANOVA test followed by a multiple comparisons procedure using Unistat for Windows v6 (www.unistat.com). A non-parametric method of analysis will be used in order to avoid any assumptions about data distribution. Differences will be considered significant if $p < 0.05$.

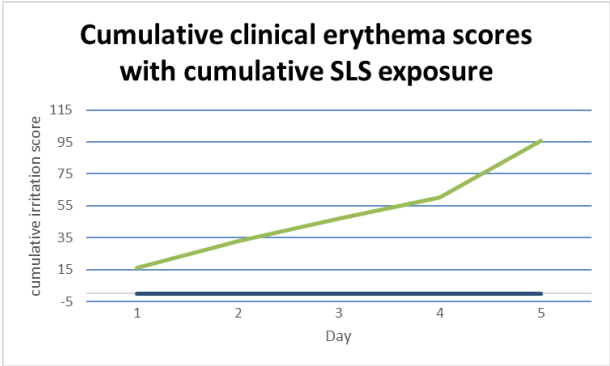
Results

Comparison of surfactant cumulative irritancy measured instrumentally and by expert assessor

The first experiments sought to determine whether SLS cumulative exposure (green line) led to cumulative irritation changes in measurable erythema, as determined by Chromameter a* values. The results are summarized below, with untreated skin as the control:



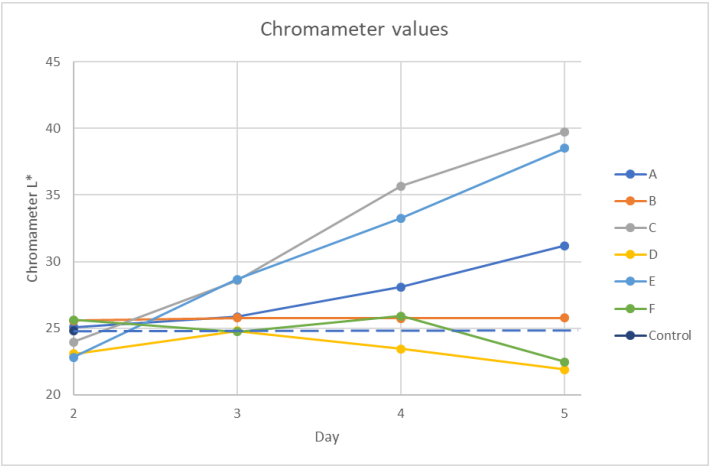
The measured erythema was also determined by expert grading of erythema by trained personell. The data from the same experiment are shown below:



Visual grading of erythema correlated very strongly with Chromameter measurements (r=0.94) indicating that expert graders can delineate degrees of erythema with high accuracy.

Ranking of cumulative irritancy of surfactant solutions

Following the initial experiments, we then compared different surfactant solutions for their irritancy potential. The results are shown below where C and E are two samples of SLS, A is SLES, B is CAPB, D and F are proprietary surfactants claimed to be exceptionally mild by the manufacturer.



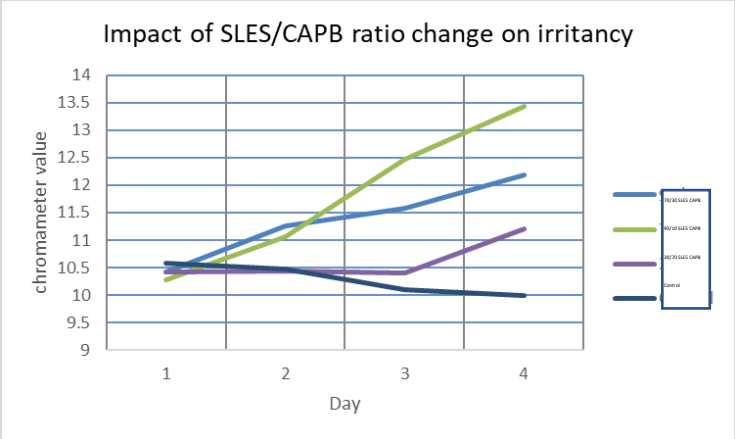
The data from these series of experiments demonstrated that surfactants could be ranked for irritation potential within 3 days of exposure and that the increase in cumulative irritation was essentially linear. The two surfactants claimed to be exceptionally mild were shown to generate essentially no erythema.

Clinical grading of the same test sites provided cumulative erythema scores that ranked the irritancy of the test materials as follows:

Rank order of mildness
Mildest
F D B A E=C

Influence of ratio of CAPB and SLES on cumulative irritation.

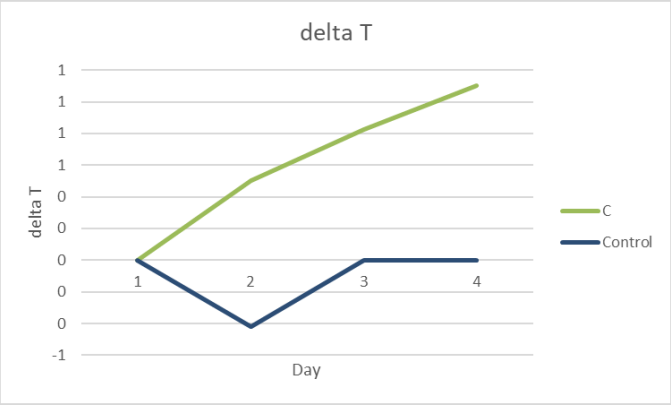
Personal care products are routinely formulated with blends of SLES and CAPB to create appropriate aesthetic qualities (e.g. foam volume) and to reduce irritation potential. We compared ratios of SLES and CAPB as follows: 90/10 SLES/CAPB, 70/30 SLES/CAPB, 30/70 SLES/CAPB in oreder to determine whether cumulative irritancy was impacted by adjusting ratios of surfactants. The results are summarized below:



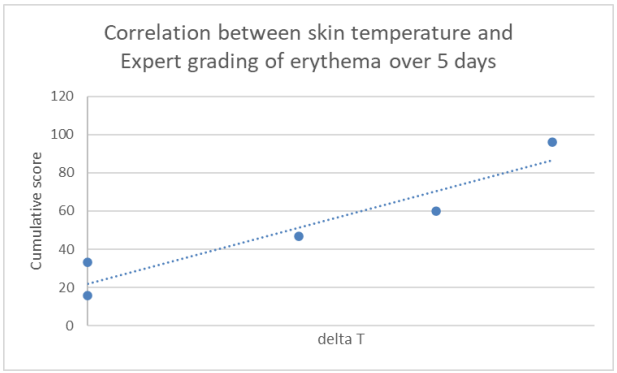
We observed that irritation potential was relative to the concentration of SLES in the blens, with 90% SLES (green line) providing the greatest erythema and 30% SLES (purple line) the lowest.

Correlation of changes in skin temperature with erythema

In order to determine whether local skin temperature could be a surrogate measure of irritation, for applications where the test material has significant colour, or stains the skin (e.g. dithranol), we measured skin temperature after application of 0.2% SLS solution, as in the above experiments. The results are shown below:



Skin temperature at the test sites was shown to associate positively with cumulative exposure to 0.2% SLS solution. We further compared skin temperature change to expert grading of the erythema observed and the results are shown below:



A positive correlation coefficient of r= 0.96 was observed between skin temperature and expert grading.

Conclusions

Determining the irritation potential of personal care products before consumers use them is vital for consumer safety and confidence. Invitro irritation assays can be performed but do not allow for cumulative exposure to products in scenarios similar to how consumers will be exposed to products, as assays are restricted to 48 hours máximum. In these experiments we have observed that expert clinical graders are able to rank the irritation potential of even very mild surfactant products and that colour and temperature measurements are potentially valuable additional measures of irritation.

The primary end point discussed in this poster is erythema. However, expert graders are able to record irritation effects that do not occur when invitro models are used, such as oedema, scaling, pustules etc. And so provide a greater understanding of the type and severity of consumer reaction to products tan can be achieved with simple invitro tests with a single end point.

We conclude that expert grading of the irritation potential of personal care products remains the gold standard for consumer protection and safety assessments.

References

[1] http://www.fda.gov/ohrms/dockets/98fr/990236Gd.pdf

[2] Dykes P J & Marks R (1992). An evaluation of the irritancy potential of povidone iodine solutions: Comparison of subjective and objective assessment techniques. *Clinical & Experimental Dermatology* 17, 246-249.