A NEW ERA FOR MILDNESS TESTING

Traditional ways of testing 'harshness' are not sensitive enough to discriminate between mild and ultra-mild formulas; but a new animal-free *in vitro* method could be the solution, write **Carol Treasure** and **Fiona Jacobs**

The World Health Organisation (WHO) has described stress as the "health epidemic of the 21st century". We know that stress puts our inflammatory responses on alert, lowering the threshold to elicit a reaction. When you take into account other contributory factors, such as air pollution or underlying skin diseases, it's no wonder that consumer demand for ever-milder products is increasing.

And, unlike some more niche claims, mildness has universal appeal across all demographics, geographies and product price ranges.

Naldi *et al*[1] completed a study of 12,377 individuals in Europe and found that 51.7% had experienced a skin reaction that lasted more than three days at some point in their lifetime. Astonishingly, 19.3% reported the same within the past 30 days and 31.8% within the last year. In addition, 37% of them avoided daily life skin care products as a result of these experiences.

While the type of skin reaction may vary, this study sends a clear message that failing to ensure the mildness of a formulation in the new decade could not only affect a consumer's experience with that product, but influence their buying decisions for years to come.

This creates a competitive environment for brands aiming to provide the very gentlest personal care products, so it's critical to have a sensitive and reproducible lab-based test, capable of picking up subtle differences.

We weren't surprised when our own market research among 33 global cosmetics brands told us that 69% of them were searching for new *in vitro* efficacy tests for mildness to skin to meet demand for increasingly mild products and ensure a competitive edge.

Research into XCellR8's XtraMild animal-free *in vitro* mildness test was funded by British government agency Innovate UK

RESEARCH AIMS

Last year in **SPC**, animal-free test lab XCellR8 reported its progress into improving *in vitro* models used to test cosmetic ingredients and formulations for mildness. As mentioned in that article, our research project aimed to:

1. Evolve and optimise existing *in vitro* skin irritation tests, with the goal of achieving sufficient sensitivity to pick up subtle differences between today's mild and mildest formulations;
2. Assess the predictive capacity of the *in vitro* test by validating directly against human patch test data;
3. Demonstrate real-life applications of the test.

STAGE ONE: TEST OPTIMISATION

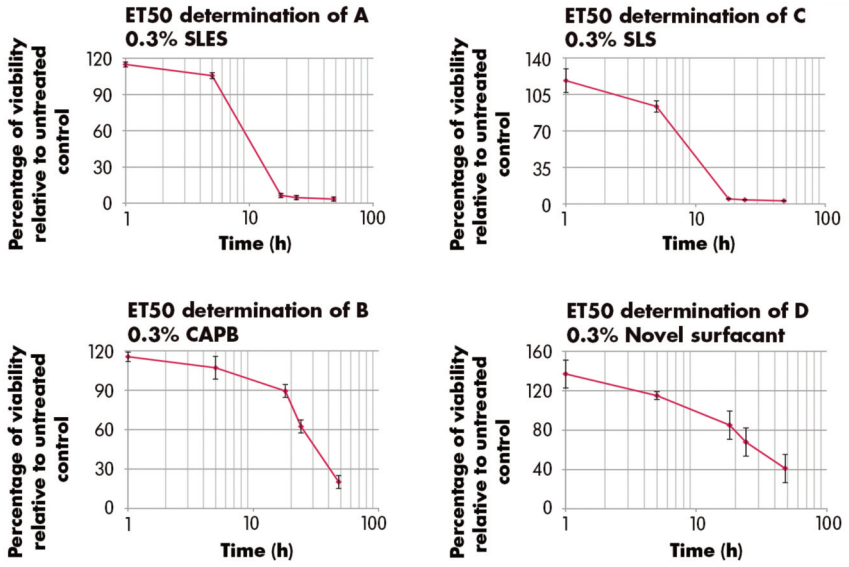
The *in vitro* test was successfully adapted from the widely used ET50 method using human reconstructed skin models. Using this approach, raw materials or formulations are applied to the skin model surface for defined time points, followed by determination of any damage to the skin cells, using an indicator of intracellular metabolism.

Test results are expressed as the ET50 value: the time taken for viability to drop to 50% of the untreated control.

In the standard skin ET50 test, the experiment is terminated at 18 hours. In our experience, many of today's new milder formulations have no impact on the skin model over this time. While this might, at first glance, seem encouraging – leading to a classification of the items as 'minimal/non-irritant' – the corresponding quoted ET50 of 'more than 18 hours' is of limited value. A range of ingredients or formulations may all return the same result, so it isn't possible to determine differences in mildness between them.

To overcome these limitations, we extended the exposure time to 48 hours, to maximise the potential to achieve a quantifiable ET50 value from the test for evaluation and benchmarking purposes. As seen in many test models, *in vivo* and *in vitro*, this may intuitively feel like a long exposure for many products, especially rinse-off personal care formulations. However, we have shown that exploiting the full capability of the

Figure 2
Assessing predictive capacity: *In vitro* and *in vivo* irritation potential of four surfactants



Test items (0.3%, pH 4.7) at 1, 5, 18, 24, 48 hrs

Rank order of irritancy: *in vitro* ET50 values and *in vivo* clinical scores (CS)

	C	>	A	>	B	>	D
ET 50	9.37		10.25		29.4		38.08
CS	14		9		4		0

Irritancy classification:
C = SLS: Moderate to Mild
A = SLES: Moderate to Mild
B = CAPB: Non-Irritant
D = Novel surfactant: Non-Irritant

Initial assessment of the predictive capacity of the test using three well-established surfactants and a novel plant-derived alternative. The same blind-coded batches of materials were tested *in vitro* and *in vivo*. All were tested at 0.3% and pH-adjusted to simulate commonly used conditions in personal care formulations. Graphs show a time course of damage to the *in vitro* skin models, leading to a calculation of the ET50 value for comparison against the *in vivo* clinical score. *In vitro* and *in vivo* methods produced closely comparable results with an identical rank order prediction of mildness in agreement with expectations for these surfactants

test system in this way proved a successful strategy in terms of superior predictive capacity, as the next section explains.

STAGE TWO: ASSESSING PREDICTIVE CAPACITY

The standard ET50 method was validated against historical animal data (Draize test). In this project, we had a unique opportunity to validate the newly optimised approach directly against human patch test data generated using the same batch of blind-coded test material. In the first phase, we used four well-known surfactants to produce a preliminary prediction model to correlate the *in vitro* data with the human *in vivo* data.

This showed that the *in vitro* system was able to obtain clear distinctions between the surfactants (three traditional surfactants and a novel non-ionic plant-derived alternative) and that the clinical scores from the *in vivo* study matched the *in vitro* predictions and rank order of mildness/irritation potential.

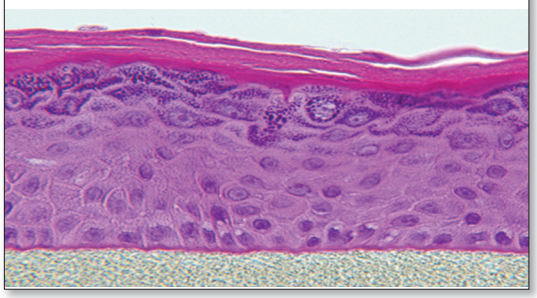
Further preliminary validation was conducted using various proportions of SLES and CAPB, and some base formulations containing the surfactants. In each case, we achieved an identical outcome in terms of rank order prediction *in vitro* and *in vivo*, even identifying the smallest subtle differences. While there's insufficient room to share all validation data in this article, we can reveal that consultation with industry partners confirmed the results agreed with expectations based on practical knowledge of the blends.

We have provided credible scientific data to show micro-differences in levels of mildness, between brands and between categories

During the project, we also went on to complete a range of funded case studies with cosmetics companies and ingredients suppliers, conducting parallel *in vitro* and *in vivo* studies on a diverse range of product groups from baby shampoos, shower gels and deodorants to face masks and moisturisers.

We were very excited as the results came through, as on each occasion our *in vitro* method had accurately predicted the *in vivo* clinical outcome, precisely matching the rank orders of irritation observed, even when the

Figure 1
Cross section of a human reconstructed skin model, manufactured and QC tested to regulatory-compliant standards to ensure reproducibility. This *in vitro* system closely models the structure and function of human skin and, importantly, incorporates a barrier function to simulate skin absorption



differences between formulations were tiny.

A study of face masks was important to include, as these products by their nature stay on the skin for a while and are prone to causing irritation in some users. The *in vitro* test was able to discriminate between the already very mild formulations, accurately predicting the rank order of the human *in vivo* clinical scores and again, matching the manufacturer's expectation based on their in-house studies.

A review of the combined results of all the validation data and case studies gave us confidence to progress with further research to expand our database of *in vitro* ET50 values for ingredients and formulations.

STAGE THREE: REAL-LIFE APPLICATIONS

Encouraged by the success of the *in vitro* and *in vivo* validation experiments and case studies, we went on to start building a database of benchmark *in vitro* ET50 values for a wide range of product groups. We saw this as a critical part of ultimately providing a valuable test for industry, so that quantifiable ET50 values could be interpreted in the context of typical industry ranges for a given product type.

BUILDING A BENCHMARKING DATABASE

In one study, we took ten soaps and 13 facial cleansers, which were a mixture of the UK's best-selling brands and consumer beauty journalists' favourites. The results were fascinating and greeted with excitement by key industry formulators.

For the first time, we have been able to provide credible scientific data to show micro-differences in levels of mildness, between brands and between categories.

While the data shows that facial cleansers overall are milder than soaps, at least three top-selling soaps were milder than their mass-market equivalent cleansers. In one case, this applied to the bar equivalent of the same brand face wash. This will come as welcome news to companies who are keen to reassure consumers who want less plastic packaging without experiencing the tight, dry feeling that can be attributed to soap.

The study also allowed us to compare the mildness of everyday value products with luxury brands, showing that product retail price isn't necessarily linked to mildness.

The benchmark database is continually evolving and we have gathered data on a diverse range of product types including baby care products, face masks, shampoos, shower gels, moisturisers, sunscreens and deodorants. We have also looked at some novel biosurfactants in comparison with standard surfactant molecules.

The test is now available from XCellR8 under the name XtraMild●

Comparing the mildness of 3 face mask formulations

***in vitro* irritancy classification**

	B>	A>	C
ET50 score	12.86	14.42	>48

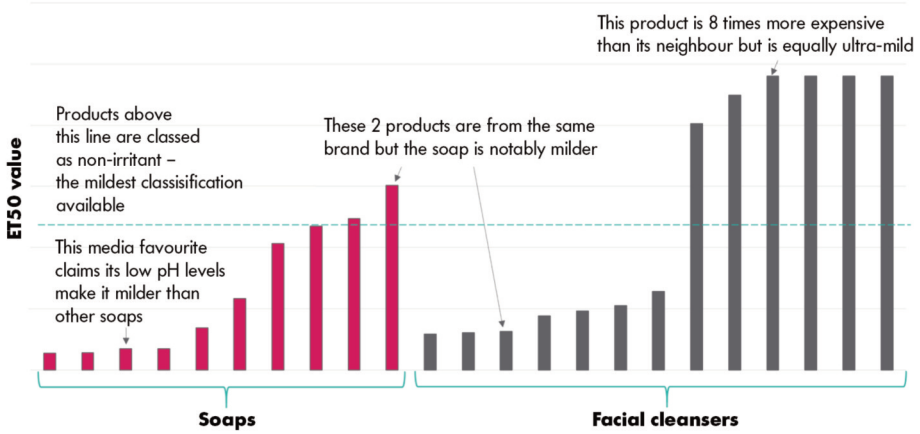
The higher the ET50 score, the milder the product

***In vivo* clinical results**

	B>	A>	C
Irritation score	11	5	2

The lower the irritation score, the milder the product

Figure 3
Relative mildness of soaps vs facial cleansers



ET50 determinations for a range of facial soaps and cleansers, contributing to a database of industry benchmark values. This was a critical part of the project, ensuring useful reference points for the interpretation of test data once the test was launched

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Reference

1. Naldi L, Cazzaniga S, Gonçalo M, Diepgen T, Bruze M, Elsner P, Coenraads PJ, Svensson Å, Bertuccio P, Ofenloch R; EDEN Fragrance Study Group. 2014. Prevalence of self-reported skin complaints and avoidance of common daily life consumer products in selected European Regions. JAMA Dermatol. 150(2):154-162.