

Safety Assessment at Unilever Applying 21st Century Toxicology



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Background & Objectives

In 2004 Unilever initiated a long-term research and capability development programme designed to evaluate and implement a new conceptual approach for assuring consumer safety without animal testing (Fentem et al., 2004; Westmoreland et al., 2010). The strategic approach and ambition of this internal programme align closely with the vision and strategy for 'Toxicity Testing in the 21st Century' published by the US National Research Council (NRC) in 2007 (Krewski et al., 2010).

Unilever's approach for safety assessment is risk-based, meaning that all available data on a new ingredient (including predicted levels of consumer exposure during product use) are used to assess the level of risk posed by its proposed consumer use. The scientific challenge we are investigating is how, in the future, novel *in vitro* data and *in silico* predictions may be used within this risk-based framework. Specifically we are investigating:

1. Risk-based approaches to assuring safety in the area of skin allergy (underpinned by a systems biology approach to understanding the mechanistic basis of skin sensitisation) (Maxwell et al., 2008; Wexwell & MacKay, 2008).
2. A case study (DNA damage-induced carcinogenicity) to evaluate the potential application of a toxicity pathways-based approach within a risk assessment context for repeat dose toxicity (Bhattacharya et al., 2011). This case study (in collaboration with the Hamner Institutes for Health Sciences, US) is designed to investigate the applicability of the principles outlined in the US NRC's report.

There is a significant scientific challenge to understand how safety may be assured for complex toxicological endpoints using data derived from a toxicity pathways-based approach that is rooted in mechanistic understanding of the underlying biology. An equally important challenge is how such toxicity pathways-based approaches could ultimately be incorporated into regulatory frameworks for the safety assessment of chemicals and products.

Safety Risk Assessment in Unilever

Unilever's evidence-based approach is built on the core principles of 'safety by design and in execution' and 'safety is non-negotiable' across its foods, home care and personal care product portfolio. All key safety concerns are identified early in the innovation process and managed pro-actively. Safety risks are assessed and managed along the R&D—Supply Chain—Consumer Use continuum (Figure 1). Safety decisions are risk-based (made on an assessment of 'acceptable risk'), taking into account the probable exposure / use of the products by consumers as well as any potential hazards and likely exposures associated with the raw materials (ingredients), product formulation and manufacturing process.

When evaluating the safety of new technologies and products, Unilever applies existing scientific knowledge and, where necessary, generates new hazard and exposure data to complete safety risk assessments. These assessments underpin decisions on risk acceptability

and any necessary risk management measures (e.g. information provided to consumers via product labels). Unilever's scientific evidence and risk assessments are included in safety dossiers submitted to regulatory authorities globally.

Scientists at Unilever's Safety & Environmental Assurance Centre (SEAC), part of the company's global R&D organisation, provide safety and risk / impact assessment expertise and guidance for the company worldwide. They are responsible for: (a) the scientific quality and transparency of assessments of consumer, occupational and environmental safety risks and environmental impacts for Unilever's key technologies, products and processes; (b) ensuring Unilever has access to the capability needed for safety risk and environmental impact assessments; and (c) developing and applying robust approaches for risk and impact assessments based on up-to-date science and in accordance with global scientific standards. The core technical expertise areas of SEAC are in toxicology and ecotoxicology, environmental science and sustainability, chemistry, microbiology and process safety, with focus on their application for hazard characterisation, exposure and risk assessments, and life cycle assessments. Many of SEAC's scientists are recognised experts in food and chemical safety, alternative approaches to animal testing, and environmental sustainability. They work collaboratively with research partners across the globe to develop new scientific capability in safety risk and environmental impact assessments.

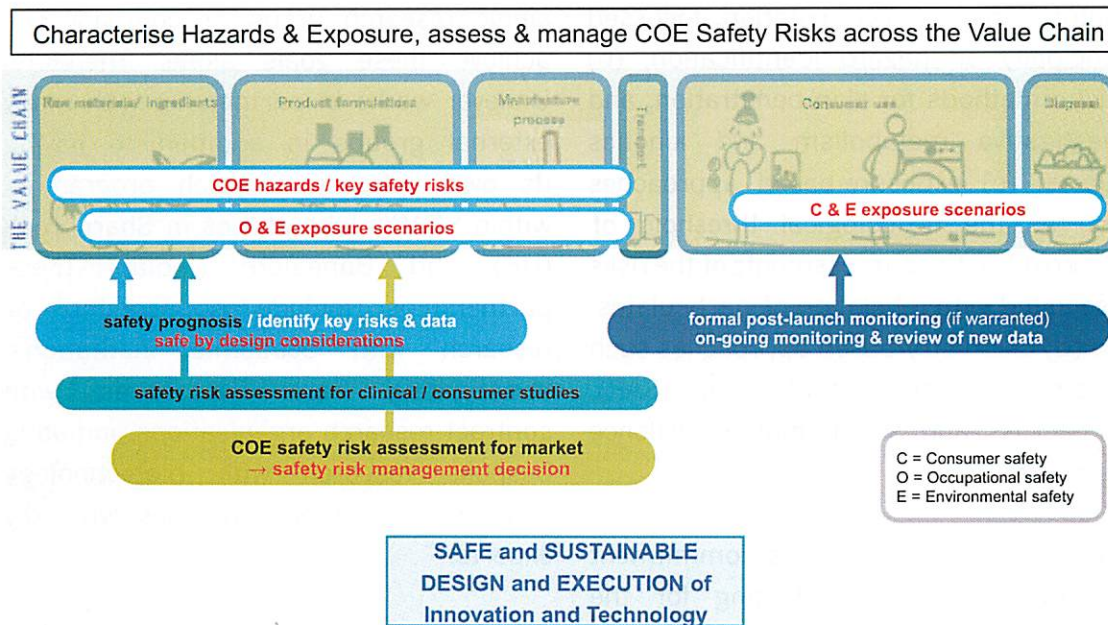


Figure 1. Safety risk assessment in Unilever; integrated approach to assessing and managing risks.

These are exciting and challenging times for toxicology as well as for other fundamental biological sciences. There is a clear modernisation agenda within toxicology, driven to some extent by the US NRC report. Advances in science and technology have the potential to transform the human health risk assessment paradigm and future chemicals risk management approaches and regulations (enabling a step change in the use of non-animal approaches and data). The strategic direction is to move away from some of the traditional hazard characterisation approaches (typically using laboratory animals) to far greater application of new cellular and molecular methodologies, and to better understanding of human biological pathways and networks and how these are affected by exposure to chemicals. Ultimately, such approaches will improve the scientific quality and

robustness of our toxicological (human health) risk assessments and risk management decisions. SEAC scientists are very active in looking to understand how best to apply the most up-to-date scientific knowledge and approaches in this area, to avoid the need to generate any new animal data within the context of Unilever's integrated, risk-based framework for safety risk assessment.

Non-Animal Approaches for Consumer Safety Risk Assessment

Over the past 20 years, SEAC scientists have established a strong scientific track record in developing and applying non-animal approaches for assessing consumer safety. This includes, for example, implementation of: (a) *in vitro* tests for skin corrosion, skin irritation,

phototoxicity and eye irritation, focussed principally on hazard identification; (b) *in vitro* methods for skin penetration, and investigative metabolism and kinetics studies; (c) exposure-based approaches such as the toxicological threshold of concern (TTC) and assessments of the risks associated with inhalation of particulates; and (d) chemistry-based approaches such as structure-activity relationship (SAR), read-across and substantial equivalence assessments.

Since the 1980s, Unilever's commitment to eliminating animal testing for the safety assessment of its products has been underpinned by a scientific research programme to develop alternatives to animal tests. From 2004 an additional €3 million a year has been invested to evaluate a new conceptual framework for an integrated, risk-based approach to the application of non-animal approaches for assuring consumer safety. Current research is focused in two main areas: (1) the development and evaluation of new risk assessment approaches for assuring consumer safety in the areas of skin allergy; and (2) a case study (DNA damage-induced carcinogenicity) to understand the potential application of a pathways-based approach to toxicity within a risk assessment context for repeat dose toxicity (in collaboration with the Hamner Institutes for Health Sciences, North Carolina, US; *thehamner.org*).

The scientific and technical challenges associated with assuring consumer safety without any animal testing are enormous and it is clear that no

single research group or company will achieve these goals alone. Therefore, Unilever works in partnership with many external groups in addition to having its own in-house research programme within SEAC's laboratories in Sharnbrook (UK) and Bangalore (India). These partnerships include sponsorship of research with academic institutions, investigating new approaches with contract research organisations, initiating bespoke research with biotechnology companies, and consultancies with key experts.

Unilever is also actively involved in EU-funded research projects; participates in cross-industry collaborative research, e.g., that conducted under the auspices of the European Cosmetics Association (COLIPA; *colipa.com*) and the European Partnership for Alternative Approaches to Animal Testing (EPAA; *epaa.eu.com*); and has ongoing involvement with other scientific initiatives on non-animal approaches to risk assessment, e.g. with the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs; *nc3rs.org.uk*) and the US-based 'Human Toxicology Project' Consortium (*htpconsortium.org*), which is looking to follow up on the NRC 'TT21C' strategy and vision from 2007 (Stephens, 2010).

In 2004 Unilever published a suggested future direction for replacing animal testing for human health risk assessment via the integrated application of new technologies and new non-animal models (Stephens, 2010; Fentem & Westmoreland, 2011). The question posed was "how

can consumer safety risk assessments benefit from applying new and emerging technologies being used in medical and biological research?" Subsequently, in partnership with other leading research teams, Unilever has invested in developing new capability in "omics" (genomics, proteomics, metabolomics), informatics (computational and mathematical approaches covering chemistry and biology), analytical, and bioengineering (tissue constructs) technologies and tools (e.g. for integrating, analysing, visualising and interpreting diverse types of non-animal data) (Westmoreland et al., 2010; Maxwell et al., 2008; Maxwell & MacKay, 2008; Fentem & Westmoreland, 2007).

The EU Scientific Committee on Consumer Safety (SCCS) published an updated opinion on the status of alternative test methods in human health safety assessment of cosmetic ingredients in the EU in 2010 (SCCS, 2010). The conclusion of the report was that the "majority of the existing alternative methods [are] only suitable for hazard identification of cosmetic ingredients and do not give information on potency. Thus, a full human health risk assessment cannot be performed." A comprehensive review, by experts selected by the European Commission, on the current scientific status of alternative methods for cosmetics testing was published recently (Adler et al., 2011). This summarises research being undertaken aimed at replacing animal tests for the key human health endpoints of: (a) repeat dose toxicity (general systemic effects, including adverse effects on target organs

such as liver, kidney, heart, etc.), (b) carcinogenicity, (c) reproductive toxicity, and (d) skin sensitisation, plus research into non-animal methods for generating toxicokinetic data. In many cases, research programmes for these endpoints are still at an early stage (e.g., SEURAT-1, the cluster of projects on repeat dose toxicity being funded jointly by the European Commission and COLIPA; seurat-1.eu).

The SCCS (2010) opinion and the outcome of the European Commission's expert review (Adler et al., 2011) indicate that in future greater priority needs to be given to developing non-animal approaches which provide biological and chemical dose-response data which can be integrated into consumer exposure and safety risk assessments. The strategic direction articulated in the US NRC report, and efforts to develop new approaches around 'adverse outcome pathways' (Schultz, 2010), offer a promising way forward in this context.

Toxicology in the 21st Century

Unilever's research over the past 8 years into new approaches to skin allergy risk assessment without animal testing aligns well with the NRC's 'TT21C' concept (MacKay, 2010). Mathematical modelling was applied to improve understanding of the mechanistic basis (biological pathways) of skin sensitisation. The insights gained then informed new research objectives. Currently, new metrics of skin sensitisation hazard are being evaluated. It is envisaged that these will enable *in vitro* data to be used for skin

allergy risk assessment rather than just for identification of the sensitisation potential (i.e. hazard) of chemicals.

Unilever's capability development projects in this area now include an evaluation of the feasibility of a TT21C pathways-based approach to safety risk assessment, in collaboration with the Hamner Institutes for Health Sciences (case study: chemical-induced DNA damage; toxicity pathways associated with DNA damage and repair). Such case studies will provide the evidence base on which to progress mapping the human toxome as proposed recently by Hartung & McBride (2011).

Like skin allergy, cancer also represents an extremely important endpoint in the safety assessment of consumer products. Past strategies for the safety assessment of DNA-reactive chemicals have relied heavily on results from *in vitro* tests (genetic toxicology tests) being confirmed by 'definitive' animal studies (genetic toxicology and carcinogenicity tests). We believe that a new non-animal strategy can be developed that is more informative and ensures safety to the consumer.

In vitro-only genetic toxicology assay strategies have a high irrelevant positive rate (i.e., positive results will be obtained for chemicals that are not carcinogenic, and many common food-based phytochemicals, e.g., flavonoids, can be rejected erroneously if *in vitro* regulatory tests are employed alone. This is because of the inherent nature of the current assays. On-going research is focussed on identifying approaches to increase the

specificity of currently available *in vitro* genetic toxicology tests. Since these tests are used purely in a hazard identification mode, the label of 'genotoxicity' indicated by the current tests necessitates the rejection of that chemical if no follow-up testing is conducted. However, greater evidence and wider acceptance of the existence of thresholds for genotoxic events, determined in *in vitro* systems, is emerging (Carmichael et al., 2009). This may provide a way forward for the risk assessment of new chemicals. High-throughput methodologies are being used, such as automated micronucleus scoring, to provide the data necessary for low-dose determinations of thresholds, in standard and newly engineered cell lines.

The characterisation of a material as 'low-dose thresholded' will require adequate understanding of the molecular mechanism of action of carcinogens, and dose-response information regarding changes in the toxicity pathways involved. 'Omics technologies offer opportunities to produce data needed to generate this understanding. Successes with transcriptomics and metabolomics have shown discrimination between chemicals with probable thresholded characteristics, based on mechanistic understandings (e.g., the activation of DNA repair pathways, changes in the cell cycle and oxidative/metabolic stress). It is hoped that novel insights that are currently being generated will be capable of informing a new risk-based approach. Several other new technologies are also being investigated, to increase our understanding of the complex interactions that occur

in biological systems in response to carcinogenic chemicals. For example, infra-red micro-spectroscopy is proving to be valuable in mapping and understanding the transformation of Syrian Hamster Embryo cells in culture, in response to chemical carcinogen exposure.

The challenge ahead is to integrate the dose-response data obtained *in vitro* from the toxicity pathways associated with chemical-induced DNA damage with information on consumer exposure and modelling of subsequent systemic exposure (e.g., using physiologically based kinetic modelling), to enable risk assessments to be performed for new chemicals in consumer products under the conditions of use. The application of systems biology approaches to anchor these *in vitro* measurements to relevant biomarkers and pathology (adverse outcome) pathways will be core to this approach.

Next Steps

The results from Unilever's research programmes in skin allergy and chemical-induced DNA damage confirm our belief that an essential element for success is the involvement of multidisciplinary teams able to draw upon new developments in multiple science and technology fields. For example, our progress in the area of skin allergy is a result of successful teamwork between chemists, immunologists, cell biologists, mathematical modellers and bioinformaticians within Unilever, and across our network of external partners. SEAC scientists are now looking to help

build effective bridges between EU and US research initiatives, to engage Chinese scientists in this research, and to ensure full alignment with TT21C concepts that recognise the importance of exposure (dose-response) data and the application of data derived from new technologies within a risk-based safety decision-making framework. In March 2011, the opportunities that the TT21C vision and strategy offer for a new approach to assuring safety without the generation of animal data were discussed at a meeting organised by Unilever at its R&D laboratory in Shanghai (China). Some of the authors of the US NRC report met with leading Chinese scientists and regulators, and scientists from Unilever to start to plan how China participates with TT21C research and education initiatives (tt21c.org).

While the key scientific challenge is to actively pursue new research objectives clearly aligned to understanding biological pathways and outcomes critical for human health risk assessments (here a clear roadmap would help increased international scientific collaboration and focus), an equally important challenge for the future is how, if successful, such toxicity pathways-based approaches could ultimately be incorporated into regulatory frameworks for the safety assessment of chemicals and products. Traditionally, *in vitro* hazard identification tests (e.g. for skin irritation) have been validated as 1 for 1 replacements for an existing animal test, according to OECD, ECVAM and ICCVAM guidelines. However, it is likely that a pathways-based approach will involve a

'toolbox' of non-animal methods, none of which by themselves will be a replacement for a current animal test. Thus, tests based on understanding toxicity pathways for use in safety risk assessments will need to be evaluated for regulatory use by a different approach. US EPA scientists have taken the initiative in responding to this challenge, and are leading discussions on how best to validate high throughput pathway-based assays that are being evaluated under the Tox21 programme.

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References

Adler S, Basketter D, Creton S et al. Alternative (non-animal) methods for cosmetics testing: current status and future prospects – 2010. *Arch Toxicol.* 2011; 85, 367-485.

Bhattacharya S, Zhang Q, Carmichael PL, et al. Toxicity testing in the 21st century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. *PLoS ONE.* 2011; 6, e20887 doi: 10.1371/journal.pone.0020887

Carmichael P, Kirsch-Volders M, Vrijhof H. Thresholds for genotoxins and their application in risk assessment. *Mutat Res.* 2009; 678, 71.

Fentem J, Chamberlain M, Sangster B. The feasibility of replacing animal testing for assessing consumer safety: a suggested future direction. *Altern Lab Anim.* 2004; 32, 617-23.

Fentem J, Westmoreland C. Risk Assessment + New Technologies: opportunities to assure safety without animal testing and better protect public health? Website <http://alttox.org/ttrc/overarching-challenges/way-forward/fentem-westmoreland>, 2011 (accessed 20 June 2011).

Hartung T, McBride M. Food for thought ... on mapping the human toxome. *ALTEX* 2011; 28, 83-93.

Krewski D, Acosta D Jr, Andersen M, et al. Toxicity testing in the 21st century: a vision and a strategy. *J Toxicol Environ Hlth B.* 2010; 13, 51-138.

MacKay C. Unilever's Skin Sensitisation Program. 2010. Website <http://htpconsortium.files.wordpress.com/2010/11/mackay1.pdf> (accessed 20 June 2011).

Maxwell G, Aleksic M, Aptula A, et al. Assuring consumer safety without animal testing: a feasibility case study for skin sensitisation. *Altern Lab Anim.* 2008; 36, 557-68.

Maxwell G, MacKay C. Application of a systems biology approach for skin sensitisation risk assessment. *Altern Lab Anim.* 2008; 36, 521-56.

SCCS. Memorandum on alternative test methods in human health safety assessment of cosmetic ingredients in the European Union, 2010. Website http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_001.pdf (accessed 20 June 2011).

Schultz TW. Adverse outcome pathways: a way of linking chemical structure to in vivo toxicological hazards, in: Cronin MTD, Madden JC (Eds.), *Issues in Toxicology No. 7. In silico toxicology: principles and applications.* Royal Society of Chemistry, UK, 2010, pp. 351-76.

Stephens ML. An animal protection perspective on 21st century toxicology. *J Toxicol Environ Health B.* 2010; 13, 291-98.

Westmoreland C, Carmichael P, Dent M, et al. Assuring safety without animal testing: Unilever's ongoing research programme to deliver novel ways to assure consumer safety. *ALTEX* 2010; 27, 61-65.