

# Comment

## The Feasibility of Replacing Animal Testing for Assessing Consumer Safety: A Suggested Future Direction

Julia Fentem, Mark Chamberlain and Bart Sangster

Safety & Environmental Assurance Centre, Unilever Colworth Laboratory, Sharnbrook, Bedfordshire, UK

**Summary** — At present, we are unable to use much of the data derived from alternative (non-animal) tests for human health risk assessment. This brief *Comment* outlines why it is plausible that new paradigms could be developed to enable risk assessment to support consumer safety decisions, without the need to generate data in animal tests. The availability of technologies that did not exist 10 years ago makes this new approach possible. The approach is based on the concept that data and information derived from applying existing and new technologies to non-animal models can be interpreted in terms of harm and disease in man. A prerequisite is that similar data and information generated in a clinical setting are available to permit this "translation". The incorporation of this additional translation step should make it possible to use data and information generated in non-animal models as inputs to risk assessment. The new technologies include genomics, transcriptomics, proteomics and metabonomics. Their application to *in vitro* and human "models" enables large amounts of data to be generated very quickly. The processing, interpretation and translation of these data need to be supported by powerful informatics capabilities and statistical tools. The use of integrated "systems biology" approaches will further support the interpretation by providing better understanding of the underlying biological complexity and mechanisms of toxicity. Clinical medicine is using the opportunities offered by the new 'omics' technologies to advance the understanding of disease. The application of these technologies in clinical medicine will generate massive amounts of data that will need processing and interpretation to allow clinicians to better diagnose disease and understand the patients' responses to therapeutic interventions. Support from clinical epidemiology will be essential. If these data and information can be made generally accessible in an ethical and legal way, they should also permit the "translation" of experimental non-animal data, so that they can then be used in risk assessment.

**Key words:** *alternatives, animal testing, clinical data, consumer safety, cosmetic testing, genomics, in vitro, proteomics, risk assessment, Three Rs.*

**Address for correspondence:** J.H. Fentem, SEAC, Unilever Colworth Laboratory, Sharnbrook, Bedfordshire MK44 1LQ, UK.  
E-mail: [Julia.Fentem@unilever.com](mailto:Julia.Fentem@unilever.com)

### Introduction

Products that are sold to consumers must be safe for them to use. Most countries have laws that confirm this principle and rightfully hold the manufacturer or the retailer responsible. In some cases, there are more-elaborate regulatory systems to ensure safety. These often include the need for pre-market regulatory approval, such as for medicines, pesticides and functional foods.

Innovation in terms of consumer products may involve the introduction of new active ingredients, or the delivery of products in new ways, which could also include packaging. The safety of all of these new materials, or new product formats, needs to be assessed. In many cases, animal data are an integral part of the safety evaluation process.

Animal testing is a subject that often generates heated debate, since there are many different views

in society as to whether or not animal testing can be morally justified. Governments, academic institutions, non-governmental organisations (NGOs), trade associations and individual companies have positions and policies explaining their views and practices regarding the use of animals for testing.

Recently, the European Union (EU) introduced the Seventh Amendment to the Cosmetics Directive (1), which includes a ban on the marketing in the EU of cosmetics with ingredients that have been tested on animals, anywhere in the world, as of 2009 (for most tests) or 2013 (for all tests). Cosmetics in this legislative context include soaps, shampoos, deodorants, antiperspirants and toothpastes, i.e. products that play an important role in personal hygiene and dental health. A consequence of the Seventh Amendment is that consumers within the EU will cease to have access to new cosmetics where innovation involves

new ingredients and when animal testing has been undertaken as part of their safety assessment. Responding to this legislative challenge requires that we should focus on developing ways to keep innovating in this category of products, while maintaining the same level of consumer safety, but without conducting animal testing.

### **Consumer Safety and Risk Assessment**

Safety, as such, does not exist. When the risk to a consumer is considered acceptable, a product is declared safe. When the risk is considered to be unacceptable, the product is considered unsafe. This explains why sometimes there are discussions about the safety of products. Whereas the manufacturer may consider a risk to consumers to be acceptable, a regulator or an NGO (for example, a consumer or environmental group) may disagree. Therefore, even in situations where there is agreement on the actual risk assessment, there may still be a dispute about a product's safety. Obviously, disputes can also arise due to different views on the way in which the assessment of the risk has been carried out.

Safety is established on the basis of a risk assessment in which hazards are identified and risks are characterised. This allows a risk management decision; the risks can be accepted or not (i.e. the product or ingredient is safe or not). Often, this includes a decision on how the risk can/has to be managed (for example, through advisory labelling on packs).

What are the risks that need to be assessed in order to take a risk management decision about the (un)safety of a consumer product containing a novel ingredient? Bearing in mind that risk is a function of hazard, the likelihood of exposure and the probability of specific events occurring, what are the hazards that are being considered and what are the probabilities? Since safety is about preventing harm and disease in consumers, it could be argued that it is necessary to exclude all conditions explained in textbooks of medicine and surgery. It is obvious that current practice is different and does not painstakingly tick off the absence of all medical conditions known to date. Nonetheless, current practice seems to be able to provide adequate consumer protection. So how is safety achieved?

When an ingredient is considered for use in a consumer product, this will be because of a certain functionality. This functionality is a starting point for an assessment of possible unwanted effects in consumers. The molecule's structure is compared with those of other molecules whose (un)safety for man is known. However, if this does not result in clarity regarding its possible effects, relevant information is sought by using selected non-animal test methods (2, 3).

### **Animal tests**

The principles of animal studies are simple. Several groups, usually of equal size, of animals are exposed to the test material for a defined period. Each group receives a different (sometimes daily) dose of the test material, except for an untreated control group that is kept under the same conditions as the exposed groups. (Sometimes, another, treated, control group may receive only the solvent vehicle in which the test material is dissolved or dispersed.) The doses between the groups usually differ exponentially. The animals are observed, and samples may be taken and/or functional tests carried out. Animals that die during the experiment are autopsied. At the end of the experiment, all the animals are sacrificed and autopsied, and usually several tests on blood and urine are carried out. The next step is to compare the findings in the treated groups with the control group. Differences are interpreted as relevant or not. The highest dose at which there are no adverse effects observed in the treated animals when compared with the control group, is termed the "no observed adverse effect level" (NOAEL). Animal studies may also be done for reasons other than to establish an NOAEL; for example, to establish the absorption, distribution, metabolism and excretion of a test material. This information can further add to a risk assessment by improving its relevance.

The traditional risk assessment process starts from the NOAEL. Exposure of the consumer is assessed from the amount of the ingredient in the proposed product and the use of the product, considering the route of exposure (ingestion, dermal contact, inhalation). Doses are expressed in weight per kilogram body weight. This consumer exposure dose is compared with the NOAEL in the animal (usually the rat); then it is adjusted for species differences between humans and rats, and for the presence of susceptible groups in the human population (4-6). In practice, this "reference" dose is 1:100 to 1:1000 of the NOAEL in the animal. When human exposure is below the extrapolated reference dose, a risk management decision is taken: the ingredient in this application (i.e. in this product) is "safe". When human exposure is higher, in practice it is considered "unsafe".

### **Animal testing and clinical medicine: similarities**

The underlying biology in the experimental animal and the human is assumed to be comparable, and common approaches and technologies are used in both toxicological studies in laboratory animals and in clinical medicine. Thus, the autopsy of the animals is very similar to an autopsy in a human that is carried out to confirm a clinical diagnosis made before

the patient died, or to understand what caused death in either a clinical or a forensic setting. Such an autopsy is undertaken in a systematic way. First, the body is inspected externally, then each organ is inspected and weighed, and a biopsy is taken for further microscopic examination. Blood, urine, bile, gastric content and cerebrospinal fluid are collected for further (chemical) analysis. In animal testing, the same procedure is followed. The same histological technologies are used, and the same biochemical analyses are carried out, often using the same type of equipment. In humans, establishing a cause of death is the outcome of the autopsy, whereas in animal testing an additional objective is to identify a difference between exposed groups and a control group, in order to establish a NOAEL.

In consumer safety terms, the control animal group is considered healthy. The treated groups that do not differ from the control group are therefore considered healthy and without harm, notwithstanding their exposure to the test material. One reason why we can afford to draw that conclusion is that the procedures and the technology used to autopsy an experimental animal are identical to those used to assess health and disease in humans. We recognise the organs, their macroscopic and microscopic appearances, and the haematological and biochemical parameters, as being relevant for humans. The fact that we can describe abnormalities in terms of human diseases known from clinical medicine, leads to comfort in concluding that "no difference combined with an appropriate safety factor" establishes a safe dose for humans.

### Animal tests and alternatives

Since the 1960s, there has been an increasing interest in developing alternatives to animal testing. The term "alternatives" encompasses the Three Rs of *reduction, refinement and replacement* (7-9), on which EU and national animal protection laws are based (10). Animal tests were traditionally carried out for: a) clinical diagnostic purposes; b) assessments of safety and efficacy; and c) research purposes (to understand biological mechanisms, etc.). Most use of animals for diagnostic purposes has now been replaced by other methods, typically chemical and immunological tests (9). The main drivers for their replacement were improved sensitivity, specificity, throughput, speed and cost.

A fundamental difference between animal testing to assess consumer safety and animal research to advance science, is that in the former case, protecting human health is the primary objective, whereas in the latter case, the interest is to better understand physiology, biochemistry, etc. in the experimental animal. In animal testing for human safety, the animal is used as a model or surrogate for humans (9), and the observations in the test ani-

mals are interpreted in terms of probable effects on human health. This approach allows data generated in animals to serve as inputs in a human health risk assessment, as described previously. To date, however, it has proved problematic to use hazard data generated in replacement alternative tests for risk assessment purposes, since it is difficult to interpret these data in terms of disease or harm in humans (11, 12). One reason for this is that there are no clinical equivalents to these types of data.

### New Technologies and New Models

Technologies are used to generate data. In clinical medicine, several technologies are used to generate data in a patient with a known disease or who is suspected of being ill. The results of the tests are compared with control or reference values generated in healthy individuals. By comparing the two and interpreting the difference, information is generated that enables the physician to make a diagnosis or to decide on therapy. In practice, this is not based on the result from a single test, but on interpreting information derived from several tests used in conjunction.

Many different technologies are used in clinical medicine, the oldest being the recording of an individual's medical history and doing a physical examination. Today, many other technologies are available, ranging from X-ray, ultrasound, various other imaging techniques and endoscopy to those based on biochemistry, electrophysiology, etc. Recently, technologies such as genomics, proteomics, transcriptomics and metabonomics have been introduced (13-17). "Genomics" refers to the study of the complete set of genes of an organism, cell or organelle, whereas "proteomics" is the study of the entire protein complement expressed by a genome, tissue, cell, etc. The term "transcriptomics" is used to describe the study of the full complement of activated genes, mRNAs or transcripts in a specific tissue at a particular time. "Metabonomics" can be described as the measurement of low molecular weight metabolites in a cell at a particular time and under specific environmental conditions. The place of the "omics" in clinical medicine still needs to be fully established, but it is obvious that these technologies generate information that will add value to the older technologies for understanding what is happening in the patient, thereby improving the making of a diagnosis and the refining of therapeutic interventions. It is also very likely that these technologies will be welcomed as value adding tools in occupational medicine and epidemiology, for example, given their role in identifying potential new biomarkers.

Similarly, many different technologies are used in experimental biology to generate data in test systems or models (for example, laboratory animals,

reconstructed tissues, cell lines, sub-cellular fractions). By comparing the data from models that have been exposed to a test material with data from the non-exposed model, information on the differences caused by exposure can be established. As in medicine, the new DNA-based and other “omics” technologies will be used increasingly (12, 18–20).

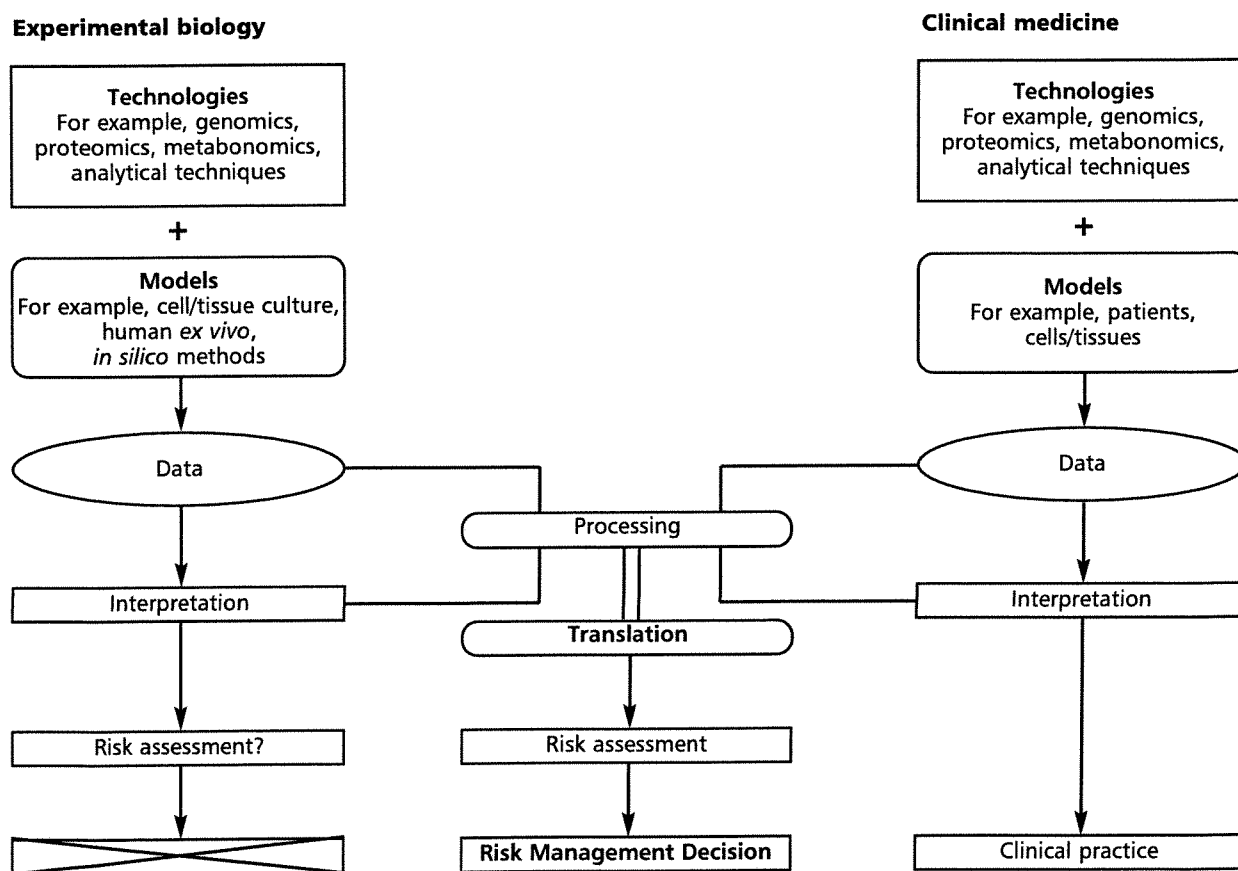
### Future directions

In the EU, innovation via the use of new ingredients in the cosmetics sector cannot be based on animal testing after 2009/2013 (1). Therefore, the question to be addressed is what technologies and “non-animal” models are available, or need to be developed, to generate the data and information

required. Doing this as a theoretical exercise generates “technology–model–data combinations” (i.e. sets of results obtained in a series of experiments in which different technologies are applied to a variety of defined biological models). It is proposed that understanding the analogies between these “combinations” derived from non-animal biological experiments and clinical studies (where the health implication of the data is known), should enable the experimental data to be “translated” (by providing appropriate informational context) and thereby used as inputs to risk assessments (Figure 1).

The data generated by such combinations may be single values (for example, concentrations) or, more typically, will be relatively complex. Often, the results obtained require considerable processing and interpretation before individual data can be

**Figure 1: A proposed new approach for assessing consumer safety without animal testing**



*The approach is based on the concept that data derived from applying existing and new technologies to non-animal models can be used as inputs to risk assessments. A prerequisite is that similar data generated in a clinical setting are available to allow the necessary “translation” to support risk assessment and a risk management decision. The processing, interpretation and translation of the large amounts of data generated need to be supported by powerful informatics capabilities and statistical tools. Using integrated “systems biology” approaches will further support the interpretation, by providing better understanding of the underlying biological complexity.*

compared with relevant controls and turned into useful information. The new “omics” technologies — genomics, transcriptomics, proteomics and metabolomics — generate large data sets of a complex nature (21). For taking a decision about consumer safety, data about individual genes, proteins and/or metabolites may be of little significance on their own. Rather, it is the insights derived from interpreting them in conjunction with other information that will be important. Thus, complex patterns and differences between these patterns will need to be processed, interpreted and translated by comparing them with similarly complex patterns from clinical medicine that are disease-related. Informatics capabilities and advanced statistical modelling approaches and tools will make possible what was impossible 10 years ago.

Given the nature of the technologies that will be deployed in the future, it seems unlikely that the information ultimately generated will allow the establishment of “simple” NOAELs which are the typical read-outs of many current animal tests. It will be more likely to generate probabilities with defined levels of uncertainty that could serve as inputs in the risk assessments of the future. To support this, new prediction models will need to be developed that will have to be based on effects that are quantified and the probability that these effects will occur. As a consequence, the paradigms that are needed to manage safety without the use of experimental animal data will have to follow approaches that incorporate a better and broader understanding of the overall biological complexity of living organisms; this is in contrast to the current paradigms and approaches, which are typically reductionistic in nature (for example, hierarchical testing strategies; 22–25).

Humans and animals are highly complex biological systems. Their biochemistry, organisation and functioning are carefully structured and highly integrated. They have been fashioned by evolution to cope with external insults. The overall vital function of the organism may be at risk only when key structures, functions and biochemical processes are affected. In other words, only when key “hubs” in the biological system are affected significantly, will disease or death result. “Systems Biology”, a term being used to describe integrated approaches to studying biological processes (21, 26–28), should enable us to understand what changes in the biological systems are underlying disease. Similarly, studying changes induced by biological, physical or chemical agents in these complex biological networks, by comparing experimentally generated data with clinical data, should enable us to predict the possible consequences for human health. Understanding and interpreting the effects observed in terms of both changes in key biological networks and causative changes in disease, should enable the complexity associated with use of the new technolo-

gies to be simplified. In turn, this should support the development of new paradigms for consumer safety decision making.

Thus, to overcome the limitation of not being able to interpret much of the data derived from the current alternative (non-animal) tests for human health risk assessment purposes, a new direction is suggested. The approach is based on the concept that data and information derived from applying existing and new technologies to non-animal models can be interpreted in terms of harm and disease in man. A prerequisite is that similar data and information generated in a clinical setting are available to allow this “translation”. These clinical data would need to be made accessible for such purposes in an ethical and legal manner. The incorporation of this additional translation step should make it possible to use data and information generated in non-animal models as inputs to risk assessments (Figure 1).

## Conclusions

It seems plausible that there will be a future in which consumer safety can be delivered without animal testing. The plausibility is derived from two sources: a) since the objective is preserving health and preventing disease in humans, it can be argued that effects in animals are of little consequence for humans *per se*; and b) modern scientific advances will afford radical insights into the functioning of biological systems, enabling the selection or design of test systems and approaches of more relevance, and better predictive value, for humans.

The alternative methods available to date have been effective in refining and reducing animal testing (11, 29–31). Replacing animal tests is much more challenging (7, 8, 24, 32, 33). The reason is probably that integrating and interpreting effects in alternative models in terms of human health has proven to be very difficult (11, 12, 22). It is not evident how the data generated in the various alternative methods *currently* available can be used in making risk-based safety decisions in the future.

The rapid increase in the use of new technologies in the “omics” area, in clinical medicine as well as in experimental biology, supported by the availability of improved *in vitro* models of human tissues, *in silico* modelling tools, advanced statistical modelling and informatics tools, and the development of novel systems biology approaches to integrate all relevant knowledge into (computer-based) models of biological processes and responses, is for the first time opening up a window to a radical future — a *future* where observations in test systems other than laboratory animals can be processed, interpreted and translated via comparisons with relevant clinical data, such that they may be adequate as inputs into human health risk assessments

(Figure 1). This would therefore enable consumer safety decisions to be made with an acceptable level of confidence — without the use of new data generated in animal tests.

## Acknowledgement

The input of many individuals within Unilever's Safety and Environmental Assurance Centre to the on-going development of the ideas outlined in this article, is acknowledged.

## References

1. EU (2003). Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products. *Official Journal of the European Union* **L66**, 26–35.
2. Lisansky, S.G., Macmillan, R. & Dupuis, J., eds (1996). *Alternatives to Animal Testing. Proceedings of an International Scientific Conference organised by The European Cosmetic Industry*, 377pp. Newbury, Berkshire, UK: CPL Press.
3. Clark, D.G., Lisansky, S.G. & Macmillan, R., eds (1999). *Alternatives to Animal Testing II. Proceedings of the Second International Scientific Conference organised by The European Cosmetic Industry*, 294pp. Newbury, Berkshire, UK: CPL Press.
4. Renwick, A.G. & Lazarus, N.R. (1998). Human variability and noncancer risk assessment: an analysis of the default uncertainty value. *Regulatory Toxicology and Pharmacology* **27**, 3–20.
5. Renwick, A.G., Dorne, J.-L.C.M. & Walton, K. (2000). An analysis of the need for an additional uncertainty factor for infants and children. *Regulatory Toxicology and Pharmacology* **31**, 286–296.
6. IGHRC (2003). *Uncertainty Factors: Their Use in Human Health Risk Assessment by UK Government*, 69pp. Leicester, UK: MRC Institute for Environment and Health. Website <http://www.le.ac.uk/ieh/pdf/er9.pdf> (Accessed 3.12.04).
7. Balls, M., Goldberg, A.M., Fentem, J.H., Broadhead, C.L., Burch, R.L., Festing, M.F.W., Frazier, J.M., Hendriksen, C.F.M., Jennings, M., van der Kamp, M.D.O., Morton, D.B., Rowan, A.N., Russell, C., Russell, W.M.S., Spielmann, H., Stephens, M.L., Stokes, W.S., Straughan, D.W., Yager, J.D., Zurlo, J. & van Zutphen, B.F.M. (1995). The Three Rs: the way forward. The report and recommendations of ECVAM workshop 11. *ATLA* **23**, 838–866.
8. Fielder, R.J., Atterwill, C.K., Anderson, D., Boobis, A.R., Botham, P., Chamberlain, M., Combes, R., Duffy, P.A., Lewis, R.W., Lumley, C.E., Kimber, I. & Newall, D.R. (1997). BTS Working Party report on *in vitro* toxicology. *Human and Experimental Toxicology* **16**, S1–S40.
9. Russell, W.M.S. & Burch, R.L. (1959). *The Principles of Humane Experimental Technique*, 238pp. London, UK: Methuen.
10. EU (1986). Council Directive of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* **L358**, 1–29.
11. Chamberlain, M. (1997). Application of QSAR, expert systems and *in vitro* methods. In *Developments in Animal and Veterinary Sciences, Vol. 27, Animal Alternatives, Welfare and Ethics* (ed. L.F.M. van Zutphen & M. Balls), pp. 723–730. Amsterdam, The Netherlands: Elsevier.
12. Eisenbrand, G., Pool-Zobel, B., Baker, V., Balls, M., Blaauboer, B.J., Boobis, A., Carere, A., Kevekorde, S., Lhuguenot, J.-C., Pieters, R. & Kleiner, J. (2002). Methods of *in vitro* toxicology. *Food and Chemical Toxicology* **40**, 193–236.
13. Garnis, C., Buys, T.P.H. & Lam, W.L. (2004). Genetic alteration and gene expression modulation during cancer progression. *Molecular Cancer* **3**, 9. Website <http://www.molecular-cancer.com/content/3/1/9> (Accessed 7.6.04).
14. Goldstein, A.M. (2003). Changing paradigms in dermatology: proteomics. A new approach to skin disease. *Clinics in Dermatology* **21**, 370–374.
15. Hanash, S. (2003). Disease proteomics. *Nature, London* **422**, 226–232.
16. Marcotte, E.R., Srivastava, L.K. & Quirion, R. (2003). cDNA microarray and proteomic approaches in the study of brain diseases: focus on schizophrenia and Alzheimer's disease. *Pharmacology and Therapeutics* **100**, 63–74.
17. Thomas, G. & Cann, H. (2003). Irruption of genomics in the search for disease related genes. *Gut* **52**, Suppl. 2, 111–115.
18. Aardema, M.J. & MacGregor, J.T. (2002). Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies. *Mutation Research* **499**, 13–25.
19. Nicholson, J.K., Connelly, J., Lindon, J.C. & Holmes, E. (2002). Metabonomics: a platform for studying drug toxicity and gene function. *Nature Reviews in Drug Discovery* **1**, 153–161.
20. Pennie, W.D. & Kimber, I. (2002). Toxicogenomics: transcript profiling and potential application to chemical allergy. *Toxicology in Vitro* **16**, 319–326.
21. Waters, M., Boorman, G., Bushel, P., Cunningham, M., Irwin, R., Merrick, A., Olden, K., Paules, R., Selkirk, J., Stasiewicz, S., Weis, B., van Houten, B., Walker, N. & Tennant, R. (2003). Systems toxicology and the Chemical Effects in Biological Systems (CEBS) knowledge base. *Environmental Health Perspectives* **111**, 811–824.
22. Barratt, M.D., Castell, J.V., Chamberlain, M., Combes, R.D., Dearden, J.C., Fentem, J.H., Gerner, I., Giuliani, A., Gray, T.J.B., Livingstone, D.J., Provan, W.McL., Rutten, A.A.J.J.L., Verhaar, H.J.M. & Zbinden, P. (1995). The integrated use of alternative approaches for predicting toxic hazard. The report and recommendations of ECVAM workshop 8. *ATLA* **23**, 410–429.
23. Basketter, D.A. (1994). Strategic hierarchical approaches to acute toxicity testing. *Toxicology in Vitro* **8**, 855–859.
24. Robinson, M.K., Cohen, C., de Brugerolle de Fraissinette, A., Ponc, M., Whittle, E. & Fentem, J.H. (2002). Non-animal testing strategies for assessment of the skin corrosion and skin irritation potential of ingredients and finished products. *Food and Chemical Toxicology* **40**, 573–592.
25. Worth, A.P., Fentem, J.H., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J. & Liebsch, M. (1998). An evaluation of the proposed OECD testing

- strategy for skin corrosion. *ATLA* **26**, 709–720.
26. Ideker, T., Galitski, T. & Hood, L. (2001). A new approach to decoding life: systems biology. *Annual Reviews in Genomics and Human Genetics* **2**, 343–372.
  27. Peri, S., Navarro, J.D., Amanchy, R., Kristiansen, T.Z., Jonnalagadda, C.K., *et al.* (2003). Development of Human Protein Reference Database as an initial platform for approaching systems biology in humans. *Genome Research* **13**, 2363–2371.
  28. You, L. (2004). Toward computational systems biology. *Cellular Biochemistry and Biophysics* **40**, 167–184.
  29. Gerberick, G.F., Ryan, C.A., Kimber, I., Dearman, R.J., Lea, L.J. & Basketter, D.A. (2000). Local lymph node assay: validation assessment for regulatory purposes. *American Journal of Contact Dermatology* **11**, 3–18.
  30. Tichias, K., Fentem, J., Basketter, D., Botham, P., Brooker, P., Bruner, L., Evans, P., Fairhurst, S., Fassold, E., Fielder, R., Gerberick, F., Harvey, P., Koëter, H., Parsons, P., Schlede, E., Shannon, D. & Spielmann, H. (1998). Progress in toxicological testing: reduction and refinement issues. Recommendations from a joint UK Government/ECVAM meeting. *ATLA* **26**, 619–627.
  31. Worth, A.P. & Balls, M., eds (2002). Alternative (non-animal) methods for chemicals testing: current status and future prospects. A report prepared by ECVAM and the ECVAM Working Group on Chemicals. *ATLA* **30**, Suppl. 1, 125pp.
  32. Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J., Holzhütter, H.G. & 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**, 483–524.
  33. Spielmann, H., Balls, M., Dupuis, J., Pape, W.J.W., Pechovitch, G., de Silva, O., Holzhütter, H.G., Clothier, R., Desolle, P., Gerberick, F., Liebsch, M., Lovell, W.W., Maurer, T., Pfannenbecker, U., Potthast, J.M., Csato, M., Sladowski, D., Steiling, W. & Brantom, P. (1998). EU/COLIPA “*in vitro* phototoxicity” validation study, results of phase II (blind trial), part 1: the 3T3 NRU phototoxicity test. *Toxicology in Vitro* **12**, 305–327.

