ANIMAL WELFARE PANEL
2015 REPORT

EXECUTIVE SUMMARY

Shell is committed to ending the need to do testing involving animals. We strive to replace animal testing with suitable alternatives while ensuring that we can continue to innovate, and develop and maintain new, safe products and technologies.

A particular barrier to progress which we have experienced in most regions of the world is a reluctance by regulatory authorities to accept alternative methods and/or read-across strategies. In Asia, however, there are specific opportunities to build capacity on alternative testing strategies.

Our priorities for 2014 were: 1) to support research and development of alternative methods; 2) to continue to advocate and lobby policy makers to accept non-animal methods; and 3) to work through consortia to minimise the numbers of animals used in mandated testing.

In the research and development of alternative methods, we achieved the development and application of a quantitative structure-activity relationship (QSAR) model for acute fish ecotoxicity, and the assessment of alternative methods for whole effluent testing. The development of an alternative screening tool for mammalian developmental toxicity and reprotoxicity is progressing well.

We have successfully advocated the use of in-vitro models for skin and eye irritation and skin sensitisation in Asia, resulting in reduction of mandated animal testing. A reduction of animal testing is also the desired outcome of our engagements with regulators in Africa, where we advocate the use of solid-phase micro-extraction as a screening tool to determine bioaccumulation. In 2014 we not only worked in consortia for testing to comply with European Chemicals regulations, but also for the development and assessment of alternative methods. Scientific outcomes are published where possible, and a publication list is presented in this report.

In 2015 we will continue to work on the aforementioned priorities and expand our efforts to the Asia region.

INTRODUCTION

There are strong ethical, scientific and business drivers to move away from animal testing as the means to demonstrate product safety. Without this safety assurance, there is no market access or license to operate. However, we live in a strictly regulated environment where animal testing is still required to demonstrate safety of Shell products and processes.

Shell is committed to eliminating the need to do testing involving animals. We strive to replace animal testing with suitable alternatives while ensuring that we can continue to innovate, and develop and maintain new, safe products and technologies.

"Shell is one of the companies working hardest to demonstrate how they are reducing animal testing." **The Animal Welfare Panel, 2015**

Shell implements the 3Rs of animal testing (replace, reduce, refine) wherever possible while meeting legal obligations and protecting human life and the environment. Any Shell-owned or Shell-operated company must follow Shell animal testing standards when laboratory-based toxicology experiments are conducted on animals, even in those countries that have less stringent requirements.

Every year the external Animal Welfare Panel ("the panel") examines and comments on the implementation of Shell animal testing requirements. This external panel works with Shell to ensure best practice in laboratories. It also advises on Shell's external engagement supporting the development and application of the 3Rs. The membership and terms of reference of the Animal Welfare Panel are provided at the end of this report.

This document details Shell's ongoing efforts to replace, reduce and refine animal testing by progressing new and alternative testing methods, and by increasing the use of scientifically robust *in vitro* assays. The report also describes Shell's external engagement and advocacy for the use of alternative methods. An overview of animal use by Shell to assess the safety characteristics and environmental impact of its products, operations and manufacturing processes is provided at the end of this report. This report has been reviewed and approved by the panel.

SUPPORT RESEARCH AND DEVELOPMENT OF ALTERNATIVE METHODS

Development of non-animal methods for human health protection
Currently, several *in vitro* skin and eye irritation models are available. Some of these models can be used to assess the potential irritation hazard, others are only accepted as a screening tool mandating follow-up *in vivo* testing. In addition, the *in vitro* models are typically validated for single substances, while a large proportion of substances manufactured globally are complex substances or mixtures. The development of *in silico* prediction methods such as quantitative structure-activity relationships (QSARs) and *in vitro* systems are starting to form a toolbox for providing scientific justification, but more development is needed to provide adequate information to waive the currently mandatory *in vivo* studies. The current QSAR tools are limited in the data sources used to develop them and are not amenable to mixtures. The development of computational models that can be used for mixtures of chemicals would present a significant innovation in this area, broaden their application and reduce animal use.

In 2014, Shell developed a research proposal for the development of high accuracy QSARs for mixtures to bridge the data gaps for predicting skin and eye irritation. This proposal has been funded by the United Kingdom National Centre for Replacement, Reduction and Refinement of Animals in Research (NC3Rs), which awarded a £100,000 contract to KREATiS (Knowledge & Research in Environment And Toxicology in Silico) and CEHTRA (Consultancy for Environmental & Human Toxicology and Risk Assessment). The project started in 2014 and aims to complete in 2016. More information can be found at http://www.crackit.org.uk/challenge-19-qsars-mix.

New chemicals potentially used for enhanced oil recovery applications were examined for the skin sensitisation endpoint using a new OECD adverse outcome pathway (AOP) decision tree scheme. Representative carbon chain lengths and structures were selected for interpolation. Two representative assays were assessed in parallel with direct peptide reactivity assays, which cover two of the key events in the Adverse Outcome Pathway framework. These assays, in addition to the direct peptide reactivity assay, provide a model to make skin sensitisation predictions in accordance with the new OECD AOP for skin sensitisation. Experience gained with these assays – via the evaluation of new Shell enhanced oil recovery products – provides valuable insights for Shell as it continues to assess the use of the new decision tree for other products and regulatory applications.

The Shell co-sponsored NC3Rs (the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research) CRACK IT challenge is showing good progress. The aim of this challenge is the development of a screening tool for reproductive toxicity. The screening tool is pathway-based and uses species whose developmental biology is well-known, such as the zebrafish *Danio rerio*, roundworm *Caenorhabditis elegans*, and the fungus *Dictyostelium*. The project is expected to complete in 2016. More information can be found on http://www.crackit.org.uk/challenge-10-predart.

Development of non-animal methods for environmental protection
In 2014 Shell developed a more robust quantitative structure-activity relationship (QSAR) model for acute fish ecotoxicity. This was achieved by leveraging the data submitted for REACH (the European Community regulation on the registration, evaluation, authorisation and restriction of chemical substances). It resulted in an improved predictability of the existing QSAR model (Austin and Eadsforth, 2014; Austin et al., 2014, 2015).

A joint project launched in 2011 by a consortium of the US Environmental Protection Agency, the International Life Sciences Institute - Health and Environmental Sciences Institute (ILSI-HESI), Shell, and three other companies completed in 2014. The project assessed the use of fish embryos from the zebra fish and fathead minnow as an alternative to testing treated wastewater effluents for chronic aquatic toxicity using early life stage fish. The fathead minnow is used by the US National Pollutant Discharge Elimination System as one of its compliance test species. The results and final outcome of the project are reported in Villeneuve et al. (2014) and Jeffries et al. (2014, 2015).

The work identified potential for cell lines to replace fish testing, but these alternative tests must be accepted by regulators. Changes in regulation requiring fish testing for effluent permit compliance are necessary before these types of alternative tests could have any impact on fish testing numbers. This has led to the organisation of an ILSI-HESI workshop aiming to resolve how risk assessments and controls of effluents can be improved without using "protected" species. European controls do not usually use fish, unlike the US and Canada. The workshop, entitled "Concepts, Tools, and Strategies for Effluent Testing: An International Workshop" will take place in March 2016 in France and is intended to, among other things, identify opportunities to reduce reliance on animal tests in whole effluent toxicity schemes, and identify and understand barriers to implementation of new methodologies.

REGULATORY ACCEPTANCE AND USE OF NON-ANIMAL METHODS

Application of non-animal methods for human health protection

IN VITRO EYE AND SKIN IRRITATION AND SKIN SENSITISATION FOR GTL SOLVENTS

Shell maintains its licence to operate and expand into new business by complying with all applicable regulations. For example, to grow the Gas-to-Liquids (GTL) business, Shell will be required to register products in many different countries which will have their own requirements for animal testing. We optimise the testing design to comply with the various regulations world-wide and advocate read-across between the various GTL products as much as possible.

For the registration of eight GTL products in Asia, Shell was mandated to demonstrate *in vivo* skin and eye irritation and skin sensitisation data. Shell originally proposed to read across from comparable substances, supported by *in vitro* data sets for all these endpoints to meet the registration requirements. This was rejected by the authorities, however, who argued that data on the actual registered substance is a legal requirement. Based on the chemical similarity of the eight GTL solvents, animal use was reduced by 50% following a combined *in vivo* and *in vitro* testing strategy. Four GTL solvents were selected to cover the low, medium and high molecular weight range of the registered solvents, which were tested *in vivo*. In parallel all eight solvents were tested *in vitro* for the same endpoints. Subsequently the animal data were compared with the corresponding *in vitro* tests on the same substance, which indicated an excellent correlation between tests. Thus, it was demonstrated that *in vitro* data could be used to interpolate between animal data points.

Regulators accepted the approach and the eight GTL solvents were successfully registered, fulfilling requirements of local legislation by relying on in vitro read across and 50% reduction in animal use.

Application of non-animal methods for environmental protection

MICROTOX AND SOLID PHASE MICRO-EXTRACTION FOR ECOTOXICITY

We are progressing the development and application of the rapid screening tools in vitro Microtox and Solid Phase Micro-Extraction (SPME) for produced water toxicity and bioaccumulation, which are aimed at reducing the need for Whole Effluent Testing using various organisms (including fish). The screening tools have been validated using effluents from a number of offshore platforms in North Sea, Nigeria and the Netherlands. Discussion with Nigerian regulators took place in 2014 with the aim of persuading them to change their views on the need for large amount of fish for toxicity and bioaccumulation testing (according to their EGASPIN Guidelines) and adopt the Shell screening tools as a first-tier approach. It is the plan to use these assays to prioritise discharges from Shell operations globally for further attention.

A number of applications of SPME have been used by Shell scientists to confirm that certain products have a more favourable environmental profile; i.e. are less bioavailable and hence less toxic or unlikely to bioaccumulate.

For example, within the CONCAWE Ecology Group a number of studies using SPME have been carried out to screen a large number of petroleum products in a category for toxicity, with the view to then selecting only the most appropriate products (and reducing the number of products) to test (Eadsforth et al., 2015).

In addition, SPME-GC data provide the weight of evidence to support that GTL Fuel or constituents will not significantly bioaccumulate (Whale et al., 2015a) and that grease thickeners (or components thereof) are not bioavailable (and hence not toxic and not bioaccumulative) once blended within a grease matrix (Whale et al., 2015b). The latter approach has now been used to address a much wider number of additive packages with fully formulated lubricants to see whether such packages are less bioavailable.

Additional testing was required for the declassification of dodecene. Daphnia tox kits were used to provide data supporting declassification, which allowed the use of limit tests with fish instead of full dose-response testing, significantly reducing the number of fish used.

When a Shell lubricant oil used on an off-shore platform was given a classification of “black” (the worst hazard category) by the relevant regulator due to lack of data, Shell was able to generate data using advanced ecotoxicity screening tests to show the regulators that the chemical was not as “black” as previously thought – and that in fact the ecological risks of a leak were negligible. By using these screening tests, Shell was able to provide data required to have the material reclassified without the use of fish, saving approximately 400 laboratory animals.

WORK THROUGH CONSORTIA TO SHARE DATA TO REDUCE UNNECESSARY USE OF ANIMALS

To avoid test duplication, Shell conducts as much testing as possible as part of consortia. Nevertheless, Shell reports animal use on a 100%-basis (i.e. the total number of animals used by a consortium is reported). This means that the "actual" reduction in animals used by Shell is not always visible in our public reports.

"Shell strives to let the world know what is possible with the use of alternatives to encourage others to follow." **The Animal Welfare Panel, 2015**

REACH

The first REACH registration deadline for high-hazard and high-volume substances was December 1, 2010.

Shell worked largely through industry consortia to meet this registration deadline. The extensive use of read-across, trend analysis, data-sharing and toxicity prediction models, as well as exposure-based waiving, allowed Shell and its consortia partners to propose waivers for most types of animal testing in the REACH dossiers they submitted. The European Chemicals Agency (ECHA) had, in several instances, challenged the use of categories, read-across methods and the use of computer models to estimate toxicity. Shell and its industry partners continue to engage with ECHA to address any concerns with REACH dossiers. To support data-sharing, Shell and industry partners publish their scientific data and knowledge on toxicity of manufactured substances.

Examples include the publication of decades of experience with hydrocarbon solvents (Adenuga et al., 2014a, 2014b; Carrillo et al., 2013, 2014; McKee et al., 2014a, 2014b, 2015a, 2015b), emerging knowledge on the mode of action of oxygenated solvents (Borghoff et al., 2014, 2015), and novel ecotoxicology approaches for the assessment of petroleum substances (Comber et al., 2014a, 2014b; Eadsforth et al., 2014d, 2015; Leon Paumen et al., 2014a, 2014b, 2015; Redman et al., 2014a, 2014b). In addition, Shell and industry partners publish their views on how animal use for regulatory compliance could be reduced, for example by using grouping and read-across strategies (Arts et al., 2014; Patlewicz et al., 2014).

ECHA commented on several submitted dossiers that animal reproductive toxicity testing was inadequate. To avoid extensive testing, alternative testing strategies were proposed (including the use of genomics data), although these are currently not accepted.

Shell remains committed to the goals of REACH, both to demonstrate the safe use of chemicals and to reduce the use of animals in testing. Shell will continue to work with industry partners to minimise REACH testing whenever it is scientifically justified.

SHELL'S OTHER ACTIVITIES TO SUPPORT ALTERNATIVE ANIMAL TESTING METHODS

Shell is active in a number of groups whose long-term aim is to develop humane and alternative means of evaluating the health and environmental effects of oil and chemical products. Shell's current external engagement includes:

- membership of the Advisory Board of the Johns Hopkins Centre for Alternatives to Animal Testing (CAAT), providing guidance and direction to the research programmes that CAAT sponsors; Shell participates in workshops and symposia in order to be kept current with the developments of in vitro and humane science;
- participation in the European Chemical Industry Council's (Cefic) Long-Range Research Initiative, which co-ordinates industry efforts in support of the 3Rs;
- engagement with a joint European Commission-industry initiative, the European Partnership for Alternative Approaches to Animal Testing (EPAA), through Cefic;
- participation in the Regulatory Steering Group and in a task force for the development of alternative approaches to fish testing, and co-sponsor of the CRACK IT Challenge to develop a screening tool for reproductive toxicity at the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs);
- membership of the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), which supports task forces and convenes workshops to advance the science necessary to replace animal testing;
- participation by Shell scientists in forums and conferences on the application of 3R's in Europe and North America.

SHELL USE OF ANIMALS FOR TESTING IN 2014

In line with standard industry practices, Shell reports on the activities of Shell-owned and Shell-operated companies. Testing programmes that are supervised by industry consortia in which Shell participates are reported separately. Shell reports all experimental animal use on a 100%-basis (each animal is counted as Shell's even if the testing programme is undertaken by multiple companies). Test data are collected from internal sources and from reports provided by external testing laboratories.

Shell use of animals to assess the safety characteristics and environmental impact of its products, operations and manufacturing processes from 2010 to 2014 is reported in Table 1. Tests that Shell currently commissions use mainly fish and rodents, and do not involve cats, dogs or monkeys. Mandatory testing to meet regulatory requirements made up 68% of all animal use by Shell-owned and Shell-operated companies in 2014.

TABLE 1: NUMBER OF LABORATORY ANIMALS USED, 2010-2014

Animals used	Tests commissioned by	Number of animals				
		2010	2011	2012	2013	2014
Fish	Shell	38,524	33,753	30,832	44,696	61,773
Fish	Industry consortia	271	0	4,368	5,576	0
Fish	Joint ventures	4,190	11,763	4,180	10,020	20,720
Rodents	Shell	2,501	2,497	150	4,368	2591
Rodents	Industry consortia	4,411	748	7,944	5,763	3,202
Rodents	Joint ventures	0	0	0	0	0
Rabbits	Shell	9	6	9	870	40
Rabbits	Industry consortia	9	0	6	4	0
Rabbits	Joint ventures	0	0	0	0	0
Birds	Shell	0	90	0	0	0
Total		**49,915**	**48,857**	**47,489**	**71,297**	**88,326**

Notes: Industry consortia are groups of companies (including Shell) that co-operate, usually within the framework of an industry trade association, to share available data and the costs of testing programmes on particular chemicals or groups of chemicals. **Joint ventures** include companies where Shell is the operator and those companies under Shell control.

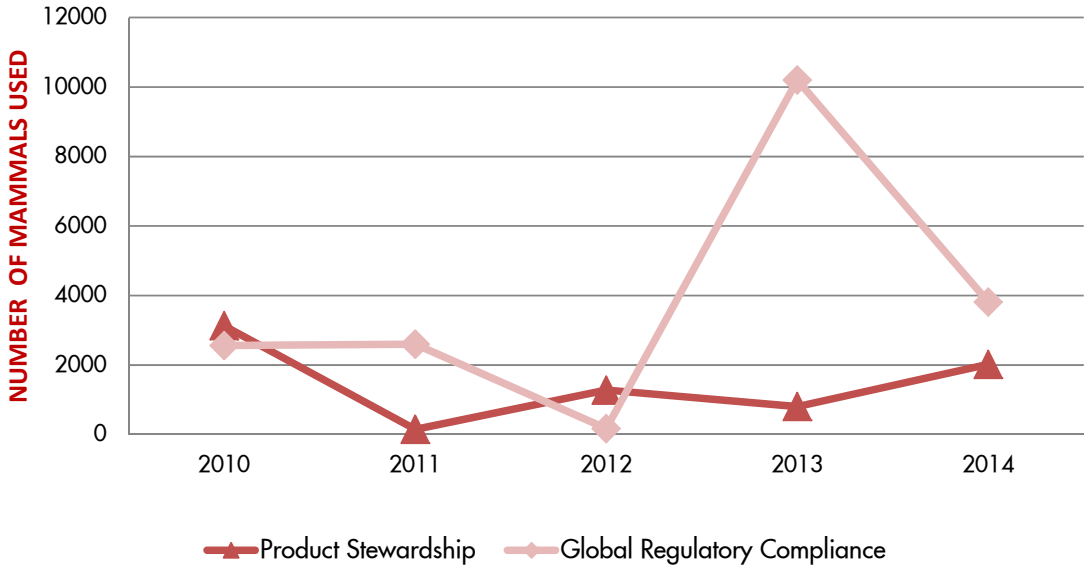
The use of mammalian species in 2014 is detailed in Table 2. Rat studies were performed to meet regulatory requirements in Europe and Asia, specifically in three prenatal developmental toxicity studies. Mice were used to assess the modes by which certain substances exert toxic effects. Rabbits and guinea pigs were mainly used to assess skin and eye irritation and skin sensitisation endpoints to meet regulatory requirements in those countries where alternative tests were not accepted. All these irritation and sensitisation tests were performed in parallel to *in vitro* testing to support read-across strategies. Shell used 5,833 mammals to assess product safety in 2014, of which 3,814 mammals were used to meet regulatory requirements. While Shell constantly strives to reduce the numbers of animals used, it also has a responsibility to take into account the statistical viability of the numbers used in order to deliver defensible and reliable results. Where appropriate, Shell involves a biostatistician to ensure the data requirements are met while using the fewest animals.

TABLE 2: MAMMALIAN SPECIES USED IN 2014

Species	Number
Rats	4,952
Mice	705
Rabbits	40
Guinea pigs	136
Total	**5,833**

The purpose of performing tests on mammalian species is illustrated in Figure 1. The figure shows the number of animals used in tests commissioned by Shell, by industry consortia, and by Shell-operated joint ventures. In general, Shell expects that animal use is likely to increase going into the future to meet the increasing requirements of the European Union's REACH regulation and other developing global regulatory agendas.

FIGURE 1: PURPOSE OF TESTING IN MAMMALIAN SPECIES



Notes: Product stewardship: Data is required to understand the health and environmental hazards of a product and is not collected for regulatory purposes directly. This may include generation of detailed information on the mechanism of toxic action. The information can be used indirectly for regulatory purposes, for example for human risk assessment and Safety Data Sheet requirements. **Regulatory compliance:** Testing is required by law.

The use of fish from 2010-2014 is summarised in Table 3. Regulatory requirements in North America were the main reason for the use of fish.

Most of the fish used for product stewardship tests were in a project to support the return of water used in Shell's oil sands operations (Dube et al., 2014).

TABLE 3: USE OF FISH, 2010-2014

Purpose of test	2010	2011	2012	2013	2014
HPV Challenge	72	0	0	0	0
Product stewardship	0	17	5,060	11,326	25,960
Regulatory compliance	42,913	45,029	34,320	48,966	56,533
Total	**42,985**	**45,516**	**39,380**	**60,292**	**82,493**

Notes: In addition to product safety testing, some countries (particularly the USA and Canada) required the use of fish to assess the toxicity of discharges into water and certain waste streams. Operating permits for industrial sites, such as oil refineries, chemical plants, supply and distribution terminals, and retail sites require the toxicity of effluent waters to be tested in a range of aquatic organisms, including fish. Table 3 also includes fish used in response to US regulatory requirements to estimate environmental hazards during site clean-up operations.

Mandatory testing of fish to meet regulatory requirements made up 64% of all animal use by Shell-owned and Shell-operated companies in 2014.

CONCLUSION

The Animal Welfare Panel has:

- critically reviewed Shell's use of animals;
- reviewed and commented on Shell's efforts to promote the 3Rs;
- discussed the implications of REACH and the new EU animal welfare directive on Shell use of animals;
- encouraged Shell to continue testing in consortia to reduce overall animal use;
- reviewed Shell internal processes to assure appropriate animal testing;
- discussed their role and their contribution; and
- complimented Shell for their commitment to the development, promotion and use of alternatives to animal testing.

PUBLICATIONS

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ABOUT THE PANEL

In 2001, Shell formalised its practices on animal testing by creating a more structured management process and by better communicating its position internally and externally. An external Animal Welfare Panel was established to provide independent scrutiny of, and support for, Shell's activities in this area.

TERMS OF REFERENCE OF THE PANEL

Individual panel members are invited by Shell to serve on the panel for a period of three years, with the possibility of being invited to serve for a second term of three more years. The panel recommends candidates who could be invited by Shell to join the panel, either as replacements for current members when their term has been completed, or to supplement the current panel membership.

The panel meets twice a year with key Shell personnel. It does not verify the accuracy of the data underlying the report. Besides assessing Shell's reporting on animal testing, the panel offers observations and advice on the company's performance with respect to the 3Rs. In recognition of their time and expertise, panel members receive an honorarium and reimbursement of travel and accommodation expenses.

PANEL MEMBERSHIP IN 2015

Charles Gentry (independent consultant on laboratory animal science), Panel Chair
Charles Gentry is a company director with international expertise in laboratory animal science. He has a specialist interest in compliance with UK and EU legislation, and in the implementation of good practice. He is a former Director and Certificate Holder under A(SP)A 1986 at the University of Cambridge, UK. Mr Gentry is Chairman of the Establishment Licence Holders Committee UK, Chairman of the Animal Health Trust Animal Welfare and Ethical Review Committee UK, Compliance consultant to the British Antarctic Survey, and a Member of the Home Office Advisory Group on Laboratory Animal Science.

Grahame Bulfield (Senior Honorary Professorial Fellow and Emeritus Professor of Genetics, University of Edinburgh, UK)
Grahame Bulfield spent the first 24 years of his career as a research geneticist. He was Chief Executive of the Roslin Institute from 1988-2002 where he transformed Roslin from a traditional farm-animal research institute to a leader in the application of modern biotechnology to animals. In 2002, he was appointed Vice-Principal of the University of Edinburgh and Head of its College of Science and Engineering. Since his retirement in 2008, he has been a non-executive director and a consultant in the life sciences sector. He has advised the UK government on animal testing and welfare issues.

Catherine Willett (Director, Regulatory Toxicology, Risk Assessment and Alternatives, the Humane Society of the United States)

Kate Willett began her career at the Massachusetts Institute of Technology as a developmental biologist studying embryology using the zebrafish as a model system and then joined a start-up company that pioneered the use of zebrafish for preclinical drug testing. Since 2006, she has focused on the science, policy and regulatory aspects of replacing animals as the basis of chemical safety assessment, first as Science Policy Advisor for People for the Ethical Treatment of Animals, and more recently at HSUS and as coordinator of the Human Toxicology Project Consortium (HumanToxicologyProject.org). She has numerous publications on non-animal approaches and advises international companies and governments on the regulatory use of non-animals methods.

Jim Bridges (Emeritus Professor of Toxicology and Environmental Health at the University of Surrey, UK)

Jim Bridges held previous positions in University of Surrey including Dean of Science and founding Head of two large health research and teaching institutes. He has published nearly 400 papers and reviews and trained 98 PhD students. He is a founder of both the British Toxicology Society and EUROTOX. Work for the EU included the Chair of the two Scientific Committees: Emerging and Newly Identified Health Risks, and Toxicity, Ecotoxicity and the Environment as well as several working groups on future risk assessment methodology that have addressed alternatives to animal testing.