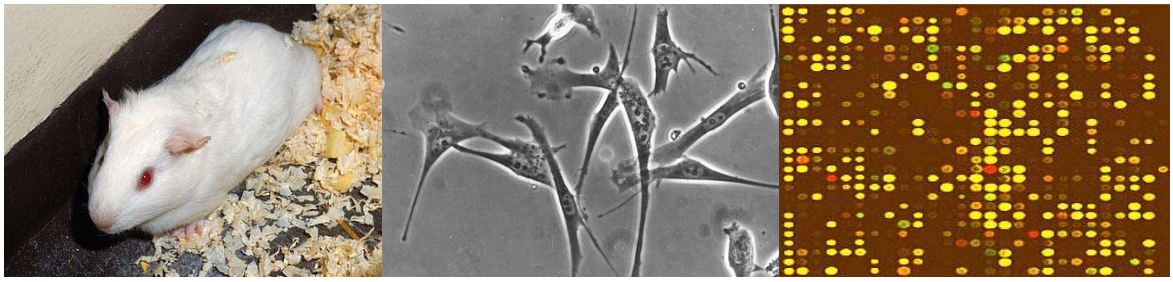
Replacement of animals in safety testing- a brighter outlook?

An RSPCA information paper



Summary

In the EU, more than a million animals are used each year in testing the safety of chemical products. More than a dozen animal test methods are used to detect potential hazards ranging from eye irritancy to birth defects and cancer.

The RSPCA has campaigned for many years for the replacement of animals in safety testing (toxicology tests) with humane, non-animal methods. Progress has been very slow, and only a few animal tests have been replaced. Toxicologists have repeatedly said that the complete replacement of animals in safety testing is not possible, at least in the foreseeable future.

However, there have been signs of a change in recent years. Public opposition to the use of animals, and demands for more and better safety assessment of chemicals, have combined with significant advances in technology to help convince people that the replacement of animal tests is not only desirable but necessary and above all, possible.

The result has been an increase in research funding for the development of replacement methods, increased activity in organisations with a role in promoting alternatives, and groups, and greater deployment of new technologies in safety testing. This report describes and analyses these developments, and assesses the prospects for accelerated progress towards the replacement of animals in safety testing.

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1. Introduction

The RSPCA has always supported the Three Rs concept: Replacement of animals in experiments, Reduction of the numbers used, and Refinement of husbandry and procedures to reduce suffering and improve welfare. The Three Rs are now firmly embedded in many areas of legislation and policy, and are supported at least in principle by a huge number of public and private bodies involved in the use of animals for scientific purposes. However, the Society's ultimate objective is the replacement of animals in research and testing with humane, non-animal methods.

The RSPCA is therefore extremely dissatisfied with the limited progress that has been made over the past few decades in replacing animals in safety testing. The latest statistics (for 2005)[1] show that in the EU alone more than a million animals are used each year to test the safety of chemicals ranging from industrial intermediates to medicinal drugs and pesticides. This number is likely to increase in the next few years, as thousands of industrial chemicals are reassessed for safety under new EU chemicals regulations (see **Changes in European laws** below).

More than a dozen different test methods are used to detect the possible hazards of chemicals, from eye irritancy to birth defects and cancer, and animals have been replaced in only a few of them, notably those for skin and eye irritancy.

Through the efforts of organisations such as RSPCA, FRAME[2] and ECVAM[3], a great deal has been achieved in avoiding animal use, for example by enforcing data sharing to avoid duplication, or using *in vitro* tests for prioritising or selecting chemicals for further development or testing. This so-called 'screening' has helped to avoid the use of animals in testing substances that will ultimately be abandoned due to excessive toxicity.

However, examples of direct replacement of individual tests are few, and scientists continue to maintain that the complete replacement of animals in safety testing is not possible, at least not in the foreseeable future. For example, the EU's Scientific Committee on Toxicity, Ecotoxicity and the

[1] Report from the Commission to the Council and the European Parliament: Fifth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union: Brussels, 5.11.2007, COM(2007) 675 final. Available at: http://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm
[2] Fund for the Replacement of Animals in Medical Experiments
[3] European Centre for the Validation of Alternative Methods

Environment (CSTEE) said in 2004 that "*for the foreseeable future, the use of live animals in toxicity testing is essential in order to perform reliable risk assessments*".[4])

In recent years there have been encouraging signs of change. Public opposition to the use of animals has combined with practical difficulties in meeting increased demands for safety testing, and with significant advances in technology, to convince people that replacement is not just desirable but necessary and above all, possible.

The result has been an increase in research funding for the development of replacement methods, increased activity in organisations with a role in alternatives development (and the formation of new ones), and greater deployment of new technologies in safety testing

In fact, scientists and their sponsors have become very publicity conscious, and they and journalists recognise that claiming to be replacing animal testing gets attention, and often improves public relations. Companies who have test methods they wish to market are the most prone to exaggerate or oversimplify, even if only in headlines.

Thus, it is easy to get a distorted view of developments from the press. For example, the Guardian of 15th February 2008 carried an article headed '**US to replace animals with robots in toxic chemical tests'**. Certainly, the involvement of robots is eye-catching, but it is only a small part of the story.

Nevertheless, there are good reasons to believe that the objective of replacing the use of animals is being taken more seriously. The development of alternatives is moving forward faster than in previous decades, even if progress is being driven more by an inability to cope with demands for testing than by a desire to save animals. Below, relevant recent developments are described, together with some of the forces driving them, and their likely impact on progress towards replacing animals in safety testing is assessed.

2. Developments within Europe

2.1. Changes in European laws

In 2003, the EU Cosmetics Directive was amended to introduce bans on the testing of cosmetics and their ingredients on animals. Deadlines were also set for banning the marketing of animal-tested cosmetics in Europe. Although such bans had been suggested before, the 7th Amendment sent a clear message to

[4] Opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on the BUAV-ECEAE report on "the way forward - action to end animal toxicity testing". 2004. Available at: http://ec.europa.eu/health/ph_risk/committees/sct/sct_opinions_en.htm

the cosmetics industry that they must replace animal use in cosmetics testing by 2009, or for some tests 2012.

An EU Regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) came into force in June 2007. This requires chemicals manufacturers to register their products, along with specified information on their potential toxic hazards. To comply with REACH, thousands of chemicals will need testing, and although the use of non-animal tests is recommended for many purposes, animal tests are still required for most chemicals. The cost of this testing will be enormous, and there are doubts about the ability of existing test facilities to undertake this amount of testing. There is therefore an urgent need for more rapid, and cheaper alternative tests to be made available both for prioritising chemicals for testing, (and the early elimination of products for which full testing might not be commercially viable), and for consideration as acceptable substitutes for animal tests in REACH.

These changes to European law have combined with the continuing public demand to replace animal tests, to provide renewed impetus for the development of non-animal test methods.

2.2. Financial support for alternatives research

A major boost to the funding of research into replacement alternatives was achieved in the EU 6th Framework Programme (2002-2007). Following lobbying by Eurogroup for Animals, among others, a number of large collaborative projects were funded which were specifically dedicated to the replacement of animals in toxicity testing. For example, projects called A-cute-tox, Reprotect, and Sens-it-iv are aimed at developing alternatives in acute toxicity, reproductive toxicology and sensitisation respectively. Other projects of various sizes, and differing degrees of dedication to replacement were also funded, giving a total investment we estimate at over €100m, of which the EU contributed €88m.

Framework Programme 7, which runs from 2007 to 2012, includes several more projects on alternatives (currently under negotiation), with EU support of over €30m. The two largest projects are based on the application of new technologies to early drug development in the pharmaceutical industry.

2.3. Test validation

The European Centre for the Validation of Alternative Methods (ECVAM) has responded to the increased legislative demand for non-animal test methods by modifying its approach to test validation, and putting more emphasis on the development of intelligent testing strategies – combinations of tests used in batteries or a step-by-step approach to safety testing.

The lengthy process of test validation has been modified in a number of ways, principally to allow new methods which are similar to existing ones to be validated by a 'fast-track' process, and promising, but not yet validated methods to be declared 'suitable' for specific purposes such as REACH registration. In early 2008, a total of 170 methods were under evaluation with 37 interlaboratory trials running.

ECVAM coordinated the European Commission's REACH Implementation Project 3.3, which developed guidance on the use of different test methods to satisfy the information requirements of REACH. The test strategies developed, which made as much use as possible of non-animal methods, have 'Revolutionised the way safety assessments for chemicals will be done in Europe in the future' according to the then Director of ECVAM, Thomas Hartung.

ECVAM has established links with similar organisations in the USA (ICCVAM) and Japan (JCVAM), to facilitate global acceptance of validated methods, although it has to be said that ICCVAM has been criticised for its slow progress.

2.4. Role of industry

The cosmetics and household products industries have been actively seeking non-animal test methods for many years, and more intensively since the passage of the 7th Amendment to the Cosmetics Directive. L'Oreal, for example, estimates that it has spent about $800m (€500m at the current rate) and Procter & Gamble has invested $225m (€145m). These sums are significant, but are small compared with the annual turnover of the companies concerned.

The pharmaceutical industry has also invested in alternative methods, although it is not greatly affected by the new legislation. This investment is driven more by the escalating costs, and diminishing success rate, of drug discovery and development. Nevertheless, the drug industry is a major user of animals in safety testing and advances such as High Throughput Screening, based on *in vitro* tests, have significantly limited animal use and provided ideas for the safety testing of other types of chemical product.

In 2005, a new European initiative was launched, bringing together representatives of the European Commission, and the cosmetics, chemicals, and pharmaceutical industries. Called the European Partnership on Alternative Approaches to Animal Testing (EPAA), the organisation has set up a number of working groups to assess current research needs, share ideas and techniques, and to find ways of accelerating the validation and regulatory acceptance of alternative methods. The EPAA is a Three Rs organisation, and does not concentrate solely on replacement, but cooperation between the EC and all relevant industry sectors should have a positive impact on replacement. However, progress to date has been somewhat slow and limited.

2.5. National programmes

A number of EU countries have unilateral programmes of research on alternatives. In general, these represent relatively small investments, and usually cover all three Rs. Thus, replacement research has not markedly increased in most countries in recent years. For completeness, however, mention should be made of the UK National Centre for the 3Rs (NC3Rs) which was set up in 2004.

The NC3Rs' mission statement begins: *'Replacement is the ultimate aim for the Centre, but as long as the use of animals continues to be necessary, every effort must be made to minimise the numbers used and improve their welfare...'*

Between 2004 and 2008, the NC3Rs received £5,525,000 from the Research Councils as well as £660,000 from the Home Office. The two Research Councils have promised to provide £12,804,000 to the NC3Rs for the period 2008-11. Other sponsors provide about £200,000 per year.

The Centre awards research grants for the development of new methods, which will increase application of the Three Rs. The amount of money committed to such projects has increased from about £1m in 2005, to £2.4m in 2007. About half of this money supports research relevant to replacing animals in safety testing.

3. The USA: a similar problem but an all-American response

In the USA, there is a growing recognition that because traditional toxicity testing approaches are time consuming and resource intensive, a large volume of existing and newly introduced chemicals cannot be adequately assessed using current testing practices.

A number of federal agencies have statutory responsibilities for obtaining and evaluating animal and human toxicity data for regulatory decision-making purposes. The numbers of health outcomes and questions that they must consider have grown over recent years, including demands for safety information on chemical effects on the endocrine system, specific risks of chemicals for children, and toxicity to the developing nervous system.

In 2004, the US National Toxicology Program (NTP) released a 'roadmap' for the future of toxicology (*A National Toxicology Program for the 21st Century: A Roadmap for the future)* in which it set out plans to use High Throughput Screening (using *in vitro* tests) conducted with the aid of robotics, into its

toxicity testing programmes. The following year, the US Environmental Protection Agency (EPA) established a National Center for Computational Toxicology (NCCT) to develop the use of computer methods in similar programmes. At the same time, the EPA and NTP together commissioned the National Research Council to develop a long-range vision for toxicity testing and a strategy for achieving it.

The NRC report was published in 2007 - *Toxicity Testing in the 21st Century: A Vision and a Strategy*. The vision is of toxicity testing carried out largely, but not entirely, without the use of animals. Assessment of chemicals would be based on the use of *in vitro* tests to detect chemical effects on specific biochemical pathways i.e. it would be primarily mechanism-based.

To implement the strategy, the EPA, NTP and the National Institutes of Health Chemical Genomics Center (NCGC) have established a collaborative research programme to pool their expertise in toxicology, computing and robotics.

Press coverage has seized upon the involvement of robotics (which is used to automate and speed up *in vitro* testing) to imply that robots will now be 'used instead of animals'. In fact, the type of robotics referred to is the same as has been in use for drug screening for years.

Ultimately, the success of this initiative will depend upon the use of valid *in vitro* tests. Both NTP and EPA have ongoing programmes of testing chemicals in a variety of such tests and NTP has already published results for 1,408 compounds in 50 cytotoxicity tests in 13 cell types. Since 2007, the EPA 'ToxCast' project has been using over 400 *in vitro* endpoints to profile the effects of 300 chemicals for which animal data exist, to see how the results correlate. The ultimate goal is to establish *in vitro* 'signatures' of *in vivo* rodent and human toxicity. To assist, computational methods are being developed that can simulate the biology of a given organ system e.g. a model of liver toxicity is currently under development in the 'Virtual Liver' project.

4. Technology to the rescue

Rapid technological progress in a number of areas, such as microengineering, information technology and robotics, has provided powerful new tools to assist the search for alternative methods of testing. Some of the ways in which they are being deployed are summarised below.

4.1. Molecular biology

One of the most promising and influential developments in recent years has been the emergence of highly efficient methods of detecting molecular changes in cells and tissues. For example, it is now possible to monitor complex changes in gene expression, protein synthesis, and levels of small molecular weight metabolites. When coupled with microchip technology and computer-assisted analysis, these methods are capable of generating huge amounts of information on what happens inside cells and tissues when they are exposed to chemicals. Because of this ability to provide a complete picture of what is happening, the methods have been given the suffix '–omic', which can be taken to mean 'relating to the whole'. The principal methods - genomics, proteomics and metabolomics – are briefly described in Appendix 1. When applied to safety testing (toxicology), these methods are generally referred to collectively as 'toxicogenomics'.

The use of toxicogenomics methods will greatly increases the potential of *in vitro* test systems to predict chemical toxicity. Instead of using single, rather crude endpoints such as cell death in cultured human cells, it is now possible to look for patterns of changes in gene expression. These can be related to the known effects of chemicals and the detection of similar patterns of response when testing new chemicals in the same cells can be used to predict toxicological effects in humans. The same approach can also be used to reduce the number of animals used in tests *in vivo*, by increasing the amount of information obtained from each animal, and allowing effects to be detected much earlier, before animals suffer severe effects of toxicity. Their sensitivity may also enable more testing to be done safely on human volunteers who may have been exposed to low levels of chemicals in the workplace.

'Omics' approaches are already widely used in biological research, and a number of large EU research projects are currently exploring the use of the methods *in vitro* as replacements for animal tests. The pharmaceutical industry is also investing large amounts of money in the application of 'Omics' techniques in drug discovery and development. In the Netherlands, €54m was allocated to the Netherlands Toxicogenomics Centre in October 2007, € 24m of which came from the Netherlands government. The amount of money invested by the US government is not yet known but is likely to be large.

4.2. Computer systems

Computers have been used to assist chemical safety assessment for many years, but it is becoming increasingly recognised that computer-assisted data analysis, molecular modelling and mathematical computation can play a vital role in replacing animal tests.

For example, the most important characteristic of the 'Omics' methods is that they provide a detailed overview of a very complex system. In fact they tend to provide so much detail that it is very difficult to discern patterns, or distinguish significant differences between large arrays of data. Advanced methods of computerised data analysis and pattern recognition have been developed to assist in the analysis of chemically induced changes.

The toxicological effects of chemicals are related to their molecular structure. By analysing existing data on the effects of chemicals, it is possible to construct computer programmes, which can to some extent predict the toxicity of new chemicals, using so-called Structure Activity Relationships (SARs). In some cases (Quantitative SARs, or QSARs), these can give information about not only which effects are likely but also their severity.

In practice, the 'domain of applicability' of a SAR is usually limited i.e. it works well only for certain types of compound and for certain endpoints, and their usefulness is entirely dependent upon the existence of accurate data on sufficient chemicals. Nevertheless, (Q) SARs are already widely used by industry to select drug candidates or prioritise chemicals for testing. The logistic problems associated with assessing the safety of vast numbers of chemicals under REACH have been a strong incentive to develop them further. The OECD released a (Q)SAR Application Toolbox in April 2008; a software application intended to be used to fill gaps in toxicity data.

Another way in which computers can help replace animals is by performing the calculations needed to bridge the gap between what a chemical does in an *in vitro* system, such as cell culture, to what might be expected in humans. There are obviously large differences between cells in a petri dish and human workers exposed to a chemical in a factory. Complex calculations must be done to adjust for the level and route of exposure of the workers, and the effects of interactions between tissues and organs within the body. Physiologically Based PharmacoKinetics (PBPK) computer modelling offers a means of making these extrapolations without the use of animals.

The models are mathematical descriptions of the complex interactions affecting the disposition of substances in the body. They are based on chemical-specific data such as blood and tissue solubility and plasma protein-binding; and species-specific physiological data such as tissue perfusion rates, fractional blood flow, and the weights of organs and tissues including kidneys, liver, fat and muscle.

Computer-based models of anatomy, physiology, and the function of specific organ systems are used as aids to education and research. Recent indications are that the concept is being expanded to try to develop models of toxicological reactions. The development of a 'virtual physiological human' seems a distant prospect, but is apparently feasible in technical terms through

the use of supercomputers or computer networking to provide the necessary processing power. There is little evidence that much progress has been made in this direction, but the idea could eventually provide a complete, computer-based means of safety testing.

4.3. Biochip technology

Further efficiency of testing can be achieved by miniaturisation, and technology applied in electronics can be used to enable many tests to be conducted simultaneously on a single chip the size of a microscope slide. For example, 'MetaChip' is a glass slide dotted with tiny (20-nanoliter) droplets of a solution containing human liver enzymes. 'DataChip' is also a glass slide, lined with droplets containing cells from the bladder, kidney or liver. The two biochips can also be used in tandem to see how the different cell types are affected by drugs subjected to metabolism by human liver enzymes.

The use of biochips requires a high-throughput microarray spotter machine to place the liquid enzyme dots on the slides and an optical assay system to detect toxicity to the cells.
Eventually, other cell types and endpoints could be introduced to increase the information obtained from the chips.

4.4. Microfluidics

One of the obstacles to the wider use of *in vitro* techniques is that they lack the complexity of the living body, particularly in terms of the interactions that occur between tissues and organs in an intact animal or human. For example, the toxicity of many chemicals depends on metabolic conversion in one tissue (usually the liver) followed by transport of the toxic metabolites to distant tissues. In addition, the biochemical activity of cells is strongly influenced by the three-dimensional arrangement of the cells within a tissue.

A partial solution to these problems is to maintain cells in enclosed chambers through which a nutrient fluid flows in a carefully controlled way. In such conditions, a more normal 3D arrangement of cells can be achieved. Furthermore, chambers containing different cell types can be interconnected by fluid channels, allowing interactions through the flow of soluble substances. The technology for producing complex, controllable, miniature microfluidic devices on silicon chips is well advanced, but will need more refinement before system any can truly be useful as a biological 'lab-on-a-chip'.

4.5. Stem cells

Stem cells retain the capacity to multiply, *in vivo* or *in vitro*, but under the right conditions will differentiate into specialised cells such as liver, nerve or muscle. Most tissues contain stem cells, but those with the greatest potential to produce different cell types are found in the embryo. Interest in the use of stem cells in toxicology is rapidly increasing. Mouse stem cells are used in one of the validated embryotoxicity tests, which detects chemical effects on the development of differentiated cell types in stem cell cultures. The use of human stem cells has a number of advantages, and efforts are currently underway to perfect culture systems in which a variety of cell types, such as liver, nerve and bone marrow, will develop and can be used for chemicals testing.

The pharmaceutical industry is particularly interested in the use of these systems in drug development. They recognise that human stem cells provide a reliable, consistent and unlimited source of cells for screening, avoiding sporadic and limited availability of human tissue, and enable better prediction of effects in humans than do animal cells.

4.6. Robotics

In vitro test methods are relatively easy to automate, and for some years the pharmaceutical industry has made use of robotics to carry out high throughput screening of potential drugs in large numbers. Of itself, robotics does not replace animal use, but it assists in increasing the rapidity, and hence attractiveness of *in vitro* approaches for chemical screening.

5. Conclusions

A number of socio-political, legislative and commercial influences have combined to encourage a more determined effort to replace the use of animals in safety testing. Recent advances in technology have opened up new and very promising possibilities for achieving this objective.

The effect of campaigns by animal welfare organisations such as the RSPCA and Eurogroup for Animals should not be underestimated. Lobbying for the test and marketing bans for cosmetics, included finally in the Cosmetics Directive in 2003, provides perhaps the clearest example of an incentive for replacement that was achieved as a result of campaigning by animal welfare organisations. Of similar importance was the campaign to include projects for the development of alternatives in the 6th EU Framework Research Programme. The drive to promote the development of alternative methods, including lobbying

for the establishment of organisations such as ECVAM and national centres for the 3Rs, has undoubtedly laid the groundwork for recent developments.

It is equally true, however, that practical considerations, and cost, are also powerful drivers of change. Confronted by increasing regulatory requirements to demonstrate the safety of a vast number of substances, it has become obvious that there are not sufficient resources in the world to carry out all the tests, if the established animal tests are used. Alongside this realisation has come a greater recognition of the scientific shortcomings of the animal tests. The chemicals industry and some regulatory bodies are turning to the new technologies for a solution to this problem. This in turn is stimulating academic scientists and commercial concerns to pay more attention to the benefits of involvement in test development.

6. Maintaining momentum

Whatever the reasons, there are signs of renewed vigour, and a change of pace in the quest for non-animal methods in safety testing. This is a cause for cautious optimism, but not complacency. The RSPCA will continue to take every opportunity to promote the development of alternative methods of testing, and to extend their use to the replacement of animals in all types of experiment. In particular, there is need for continued vigilance and pressure in the following areas:

- The 7th Amendment to the EU Cosmetics Directive promises bans on the testing of cosmetics and their ingredients, and on the marketing of cosmetics tested on animals. There is a danger that the cosmetics industry will resist these bans, particularly in those cases where non-animal tests have not yet been developed for specific types of toxicity. The RSPCA will be lobbying to ensure that the bans are not postponed again or, worse still, overturned.

  There is also doubt about how the marketing ban will be enforced. Which authorities will be responsible for enforcement, and how will they ensure that they have the necessary information on products marketed in the EU? This point needs to be pursued with European governments.

- During the passage of the REACH legislation on chemicals, Eurogroup and RSPCA won significant concessions, which should minimise animal use and encourage the more rapid development and use of non-animal test methods. For example, sharing of data and testing obligations was made mandatory, to avoid duplicate testing. Proposals to test on animals require prior approval by the authorities, and intelligent, stepwise testing strategies making full use of alternative methods are encouraged. To ensure that

these measures are effectively enforced, RSPCA will monitor and try to influence the activities of the new European Chemicals Agency, which is responsible for implementing REACH.

- Ultimately, the methods used to test products for safety are determined by legal requirements, regulatory guidelines and standardised test methods set down by bodies such as the OECD and the pharmacopoeias. In this context, replacing animal tests with alternatives methods is a continuing challenge, involving challenging the validity of the animal tests, validating and gaining timely regulatory acceptance of the alternatives. RSPCA will continue to work with organisations such as ECVAM, EPAA, ICAPO[5] and ICAPP to facilitate and accelerate this process, and will assist Eurogroup for Animals to lobby for the necessary changes in EU legislation.

- RSPCA will continue to lobby for increased financial support for research on methods to replace animal use, both in the UK and in Europe (through Eurogroup). The Society will promote the development and use of alternatives through its involvement with organisations such as FRAME, Focus on Alternatives, and the NC3Rs.

[5] The International Council on Animal Protection organisations in OECD programmes, and its sister organisation, The International Council on Animal Protection in Pharmaceutical Programmes

Appendix 1: Further information on toxicogenomics methods

Genomics: Microarrays

DNA microarrays are small, solid supports to which the DNA of a number (often thousands) of different genes are attached at fixed positions. The supports may be glass microscope slides, silicon chips or nylon membranes. The DNA is attached by printing, spotting, or direct synthesis onto the support. In this way, DNA representing each gene is located at a specific position in a grid pattern.

The level of activity of specific genes within a tissue or cell sample is determined by converting mRNA in the sample to DNA and simultaneously labelling it with a fluorescent marker. The sample DNA is then allowed to hybridise with the DNA immobilised on the microarray. The amount of fluorescence at each position on the array indicates how much mRNA was being produced from each specific gene at the time of sampling. Increases or decreases in gene activity can be detected by comparing samples from treated and untreated cells or tissues.

It is assumed that the effects of chemicals should be reflected in changes in the pattern of gene expression in affected tissues. Furthermore, chemicals with specific toxicological effects (such as cancer induction, neurotoxicity or liver damage) should cause characteristic changes. A large amount of work, using gene expression profiling, has tended to show that this is the case for chemicals causing certain types of liver toxicity, kidney damage, effects on sex hormones, cancer and skin sensitisation. These findings suggest that gene expression profiling might be a good way of predicting the hazardous properties of previously untested chemicals.

It is recognised that changes in gene expression do not tell the full story of how tissues respond to chemicals. Transcription of DNA into messenger RNA (mRNA) is only a first step in the production of proteins, which in turn have complex and profound effects on cellular metabolism. A full picture of chemical effects would include studies of proteomics and metabolomics as well as genomics (gene expression).

Proteomics

Since the 1970's, the main technique for identifying and measuring cellular proteins has been two-dimensional gel electrophoresis. Proteins are separated according to their electric charge and their mass by applying an electric current to a polyacrylamide gel, first in one direction and then at right angles to the first. Identification, by position on the gel, is rather inaccurate. A

breakthrough occurred with the development of techniques allowing mass spectroscopy to be used for identification. These are Matrix-Assisted Laser Desorption/Ionisation (MALDI) and ElectroSpray Ionisation. Using these methods, the amounts of large numbers of proteins in cells can be measured at the same time.

Metabolomics

A great deal can be learned about the functioning of living tissues by studying small molecular weight chemicals (metabolites) in tissues, or in body fluids such as blood and urine. The way in which tissue or body fluid levels of such chemicals change under different circumstances can give clues about the basis of a disease, or the effects of a drug or dietary supplement. Separating, identifying and measuring a large number of different metabolites from a single sample requires the use of sophisticated techniques such as gas or liquid chromatography linked to mass spectroscopy.

Toxicogenomics and the replacement of animals

As yet, the new methods cannot replace all animal tests. However, they have considerable potential to reduce the number and size of animal experiments, and to reduce animal suffering in some of the tests which will take longer to replace.

The use of more specific endpoints may help to improve the reliability, and hence the acceptability, of *in vitro* methods. For example, the use of cultured cells, particularly human cells, has been a very useful and often successful approach to detecting and studying toxicological effects without using animals. However, a major problem with these tests is finding a measurable effect on the cells (an endpoint) that accurately reflects toxicity to humans. Often, the effect of a chemical in cultured cells is detected as cell death, or inhibition of cell growth or respiration. It is not clear how these effects are related to properties such as skin irritancy, liver toxicity, or carcinogenicity. With the new methods, it may be possible to detect changes in cultured cells that are more directly and specifically related to a particular toxicological effect.

Ultimately, knowledge of <u>how</u> chemicals cause adverse health effects can greatly reduce the need for animal experiments. For example, knowing which molecules are the initial targets of chemical attack, and how they are altered, is of considerable use in the construction of computer-based systems for predicting toxicity on the basis of chemical structure.

Reduction in animal use

Because of the great sensitivity of the new methods, and the ability to apply them to small samples of tissues or body fluids, early indications of possible toxicity could be obtained by analysis of blood or urine samples taken from human volunteers who have received a very low and harmless dose of a chemical. This may increase the possibility of using human data instead of animal tests.

Toxicogenomics can greatly increase the amount of information obtained from each animal used in a test and thereby reduce the number of animals needed. For example, it may be possible to combine several animal tests designed to detect different toxic effects, if early signs of different types of toxicity can be identified simultaneously in tissue samples. Elimination of some of the longer-term tests may also be possible if specific early changes can be linked to the later development of specific adverse effects. This would be of particular value in pharmaceutical safety testing, where studies of different duration (notably the repeated dosing of dogs and primates for 2-4 weeks, 3-6 months and 9-12 months) are required to support human clinical trials with different treatment periods. A separate set of animals is required for each test because all are subjected to post mortem analysis at the end of treatment. If it could be shown that long-term effects can be predicted by detecting molecular changes in tissue or body fluid samples taken at appropriate intervals, only one test (the longest) would need to be initiated.

In some circumstances, particularly in the selection of suitable drug candidates, an early warning of significant toxicity is sufficient to stop further testing. Where *in vitro* screening methods are not available, a small-scale genomics experiment may be very valuable in preventing wasteful animal testing.

Lessening animal suffering

A major advantage of the early detection of markers of toxicity would be that animals need not be treated with a chemical for so long, and need not suffer the final stages of development of a chronic toxic effect such as cancer growth.

Implementing the new technologies will increase the sensitivity of detection of significant effects, and thus allow lower and more ‘realistic’ doses to be used. This could eliminate the need to use large, overtly toxic doses which cause more severe effects in the animal.