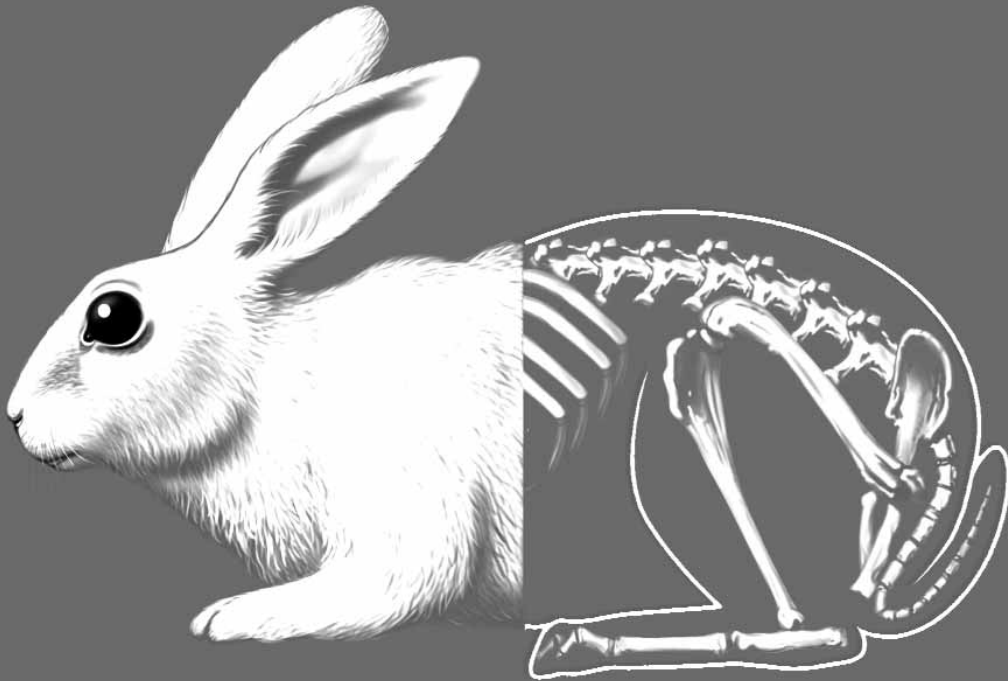


Keep animals out of REACH



**An advanced science and technology testing strategy to replace the
animal testing proposals within the REACH regulation**

Animal Defenders International
National Anti-Vivisection Society
Lord Dowding Fund for Humane Research

www.ad-international.org

A new testing and risk assessment strategy using advanced science and technology to replace the animal testing annexes within the REACH regulation

Animal Defenders International, the National Anti-Vivisection Society, and Lord Dowding Fund for Humane Research (based in the UK), have formulated a strategy to replace the animal testing regime in the EU REACH chemical regulation proposals.

Our Background:

Animal Defenders International with offices in London, South America and the U.S., was founded in 1990; represents the NAVS and LDF internationally, and works on a range of animal and conservation issues worldwide. The National Anti-Vivisection Society (NAVS), founded in 1875, is the world's first group to raise the issue of the use of animals in experiments and remains in the forefront of research and activity on the subject today. We investigate, research, and publish scientific and technical reports on the use of animals in research, as well as educational materials. The Lord Dowding Fund for Humane Research (LDF) (founded 1974) is a department of the NAVS which provides funds for scientists conducting non-animal scientific and medical research, with an annual research spend of circa £300,000 (€435,667).

The stated aims of REACH are the protection of human health and the environment, together with the promotion of non-animal testing strategies. However the current REACH proposals, relying as they do on animal tests which have not been scientifically validated, which are out of date and often adapted from different tests, and which utilise the wrong species and inappropriate doses of test substances, will not deliver any of the stated objectives of REACH.

The ADI/NAVS/LDF proposals will, we believe, significantly increase the effectiveness of REACH and prevent unnecessary animal suffering:-

- **It would replace** the animal tests detailed in REACH with modern methods, and
- **introduce fast computer screening** and *in vitro* techniques to immediately remove toxic, persistent and bioaccumulative chemicals from the market and replace with greener alternatives – avoiding the delays caused by animal testing.
- **It would set deadlines** for validation and implementation of non-animal methods.
- **It avoids problems of ensuring that data** on chemicals is shared. We have suggested a workable approach to the problem of data sharing, where initial screening for the first 3-year phase would benefit from, but not require data sharing.
- **A comprehensive database** of all known chemicals in products would be set up with contributions (worldwide) from companies, government laboratories, contract research laboratories, consumer/animal/green groups, academia, environmental monitoring agencies. It would also provide an appropriate level of public access.
- **Incentives would encourage companies** to share the data – for example by charging a fee for anyone who wants to use their data. Companies would therefore be encouraged to share data on chemicals that they do not intend to register, as well as those scheduled for registration under REACH. Thus continuing to build a central database of knowledge on all chemical substances.

- **Mandatory data sharing with substance registration:** Currently, REACH allows companies to avoid data sharing – each company undertakes a separate registration for the same substance, so a company that is last to register can avoid sharing data. Our proposals would enhance the proposals from the UK and Hungarian Governments for ‘one substance, one registration’.
- **ECVAM expanded (European Centre for the Validation of Alternative Methods),** and provided with greater funding in order to provide a sensible strategy to deal with the amount of information that is sought within a reasonable timeframe. The REACH proposals do not make any recommendations for the expansion of ECVAM, despite the stipulation that alternatives to animals should be used wherever possible. If the undertaking about replacing the use of animals is to be taken seriously, more cash must be provided, and this should come from industry as well as the EU.

Introduction:

The European Commission has proposed a new system to regulate the manufacture, import and use of chemical substances. REACH (Regulation, Evaluation and Authorisation of Chemicals) will create a new Chemicals Agency. As mentioned earlier, the aims of REACH are to protect human health and the environment, but also, to promote non-animal testing. Yet there is nothing in the proposals to replace the use of animals. Worse, it is likely to bring about the painful deaths of millions of laboratory animals, in experiments that are unreliable, unethical, and unnecessary.

We all want to see dangerous chemicals removed from our environment, but REACH will cost billions of euros and the lives of millions of animals – and the animal tests will not deliver safe chemicals for people or the environment.

The UK’s Royal Commission on Environmental Pollution (RCEP) has said of REACH: *“The infeasibility of carrying on with traditional approaches for hazard and risk assessment and not exploiting fully new technologies and advances in computational assessment techniques are serious failings.*

“Current approaches to assessing and managing risks of chemicals in the environment are cumbersome, unsound and rely heavily on animal testing. A new paradigm is needed.”

Animal test data is undermined by species differences in respect of anatomy, the structure and function of organs, metabolism of toxins, rates of detoxification and protein binding, absorption of chemicals, mechanisms of DNA repair and lifespan, and more. Variability in results is introduced not just by differences in experimental protocols but by differences in age, diet, sex, strain, genetic and immune status. Furthermore, the tests do not mimic the natural course of exposure, and what has been referred to as the ‘chemical cocktail effect’, where humans and the environment are exposed to a range of chemicals in small doses, over a long period of time. This also causes unexpected effects. Laboratory animal toxicity tests are a clumsy attempt to model this complex set of conditions and circumstances. In the laboratory, large doses of a chemical are administered in artificial conditions, over a relatively short period of time.

REACH (Registration, Evaluation, Authorisation of Chemicals):

Registration: Companies will submit data on around 30,000 substances which are produced in volumes of over 1 tonne per annum (high production volume – HPV chemicals). This information to be carried on a central database.

Evaluation: The registered information will be evaluated by the authorities. It is the testing regime at the centre of this evaluation programme which is considered to be inadequate for the task. The UK's Royal Commission on Environmental Pollution has said: *"...their use will lead to continued paralysis of the existing chemicals programme.."*

Authorisation: Substances which give rise to cause for concern would require authorisation for specific purposes regarded as safe before they can be marketed in the EU. The number of known chemicals likely to require authorisation is currently estimated at 1,400 – the list includes carcinogens, mutagens, reprotoxic compounds and persistent organic pollutants.

Existing Substances (Regulation 793/93/EEC): The European Inventory of Existing Commercial Chemical Substances (**EINECS**), lists **100,116** chemicals that were on the market in the European Community between 1971 and 1981. Regulation 793/93/EEC set up a programme designed to identify and control the risks posed by EINECS HPV substances. It has taken Member States' authorities 11 years to conduct (but not necessarily complete) risk assessments for 140 substances.

New substances (Directive 92/32/EEC): The European List of New Chemical Substances (**ELINCS**) currently holds **3,200** substances, first marketed after September 1981. Before a new substance can be placed on the market, manufacturers are required under New Substances Directive 92/32/EEC, to provide data sets of information. It is clear that companies have tended to use 'existing' substances, rather than provide information required under 'new' substances regulation¹.

The REACH proposals will introduce new regulations and management of chemical substances. However the proposal is that within the same timescale, 11 years, using the same testing methods, industry should test 30,000 substances from the EINECS list of HPV substances.

By contrast, new techniques using cell lines expressing enzymes can be used to determine metabolites (a process crucial in the determination of toxicity) so that 200 chemicals a month can be screened. The new screening techniques will make it possible to handle the variable of genetic diversity for proper risk assessment.

Animal tests are expensive to perform. For the UK alone, the bill for performing animal tests is prohibitive. On the assumption that something like 20% of the REACH bill will be borne by the UK, one estimate puts the cost of REACH to UK industry at between £250,000,000 and £930,000,000 and another has put the cost of testing at 60% of the overall figure. This means that the testing cost for the UK alone will be in the order of £150,000,000-£560,000,000¹.

The RCEP has criticised the REACH testing strategy as it stands, stating:

- it is time-consuming, expensive, and unlikely to achieve its aims;
- animal testing will delay the proper assessment of potentially lethal chemicals while cumbersome testing protocols are carried out;
- it is not realistic to expect a comprehensive risk assessment to be carried out on all the chemicals currently on the market in the timescale suggested, using the testing strategy outlined.

Eliminating dangerous chemicals

The REACH proposals arise from the acknowledgement that many chemicals have not been adequately assessed for their effects on humans and the environment. Many have not been tested for even the most basic indications of environmental hazard. Additionally, because most are sold for formulation into finished products, it isn't uncommon for chemical producers and retailers not to know what chemicals are in finished products¹.

Public concern about chemicals in the environment has deepened with revelations that chemicals have found their way into human tissue, even breast milk. Some have been shown to disrupt the hormone system (endocrine disruptors (ED)). These can disrupt male and female hormones, causing either reduced or increased hormone effects; this has been linked to rising rates of testicular and breast cancers, declining sperm quality counts, early puberty in girls, and changes in neurological development. Effects seen in animals include malformations in frogs, cancers in whales, infections in seals and polar bears, and sexual abnormalities in shellfish.

The key substances currently in our environment that have been identified as being 'chemicals of concern' include:

- carcinogens, mutagens, and substances toxic to the reproductive system;
- substances which are persistent, bioaccumulative and toxic, and those classed as very persistent and very bioaccumulative;
- endocrine disruptors.

We believe that there should be a phase-out of the worst chemicals, to be replaced with safer, greener alternatives. Some, for example, the very persistent, very bioaccumulative chemicals should be removed from the market without delay.

These chemicals are in our food and food packaging, in household cleansers, textiles, clothing, computers, toys, paints, upholstery, cosmetics, and drugs. They can even seep from washing machines, refrigerators, and televisions. 'All purpose cleaners' may contain sensitising substances which can irritate the skin, biocides to kill bacteria, etc., and volatile organic compounds (VOCs) which can irritate the eyes, throat and lungs. The production process involved in making these chemicals, and the resultant wastes, can be very toxic to fish and humans. Laundry and dishwashing detergents can contain sensitising substances and phosphates that lead to eutrophication (algae overgrowth in lakes and rivers, killing other life). Formaldehyde may be used as an anti-crease agent in textiles; it is classified as carcinogenic, poisonous, corrosive and allergenic.

For the sake of both humans and other animals, it is vital that dangerous and polluting chemicals are eradicated swiftly – but animal tests have been criticised by ecotoxicity experts as being the wrong model for this task. Other scientists agree that they will not deliver the safety information sought.

One Substance, One Registration. Data Sharing. Monitoring:

There are a number of problems within the REACH proposals which need to be tightened up. One of the most serious is that of the sharing of data. The current system does not ensure that data is shared between companies, and this could cause duplication of tests.

Currently, REACH allows each company to undertake a separate registration for the same substance, so the company that is the last to register can avoid sharing data. Companies should share data through the Substance Information Exchange Forum (SIEF), however, if a company that is a member of a SIEF refuses to share data, the other members are allowed to go ahead as if the data did not exist. This should be tightened by making the sharing of data under REACH mandatory. There should be penalties to reflect that failure to share data would be in contravention of not only the principles of the REACH regulation, but also of EU Directive 86/609/EEC on the use of animals in research.

One Substance One Registration: The UK and Hungarian governments have proposed that there should be one registration for each substance. Groups of companies would apply for registration of one chemical substance, supplying a set of data. This would avoid duplication of testing, and would ensure that as much data is collected as possible on a substance. **We support this proposal.**

Data sharing is vital to good science and effective regulation and risk management, as well as minimising animal testing. Mandatory data sharing achieves this. However, our proposal below for a technologically advanced, risk-based strategy, does not completely rely upon data sharing.

We agree with consumer and environmental groups that environmental monitoring has already highlighted chemicals of concern; most of the chemicals under discussion have been on the market for many years and so their effects on people and the environment are already known. Known hazards should be removed and replaced. For example, very persistent and very bioaccumulative (vPvBs) should simply be withdrawn with no further testing.

Such a precautionary approach would secure, as rapidly as possible, one of the prime objectives of REACH – protection of the environment and human health.

A scientifically and technologically advanced, risk-based strategy:
for the: initial screening, prioritisation, investigation, risk assessment and evaluation of chemicals – INSPIRE

Our technologically advanced, risk-based strategy is a different way of setting priorities and uses different methods to obtain information about substances. The setting of priorities in our system works well within REACH, whilst replacing the animal tests outlined.

Our strategy is also based upon a **precautionary approach**. At the same time, a reasonable plan should allow industry to substitute less hazardous chemicals, and orientate itself towards a green chemistry.

It should be borne in mind that a number of *in vitro* tests may already be sufficiently developed for identifying potential hazards, although not yet in a position to deliver a full toxicological profile.

Our technologically advanced risk-based strategy proposal consists of–

- mainly computer-based screening to identify chemicals of concern;
- a comprehensive database of known information, with input by all stakeholders
- computational and *in vitro* systems* to augment the toxicological profile
- a scheme of environmental monitoring

**Hereafter, when we refer to in vitro techniques this should be taken to include computer technology*

A number of different developments need to run in tandem if there is to be any hope of making headway with the huge number of chemicals to be tested.

According to experts on the UK's Royal Commission on Environmental Pollution (RCEP), the inability of the authorities to test even the small number of chemicals presently selected as priorities “*is largely due to the antiquated, cumbersome and expensive procedures used for hazard characterisation, which were in place 30 years ago and are still used today*”¹.

The technologically advanced risk-based strategy works on the principle of adding data to the central database as and when it becomes available, and undertaking risk assessments as these become possible. We have called this INSPIRE, but any similar strategy would work.

On the basis of predictions by the European Centre for the Validation of Alternative Methods (ECVAM), with reasonable assumptions and serious funding for alternatives, it is possible to replace existing specific animal tests with *in vitro* alternatives in the second phase of the advanced technology programme. Industry will require time to submit information identified by the initial screening programme, and this time can be utilised to fast-track acceptance of *in vitro* methods.

INSPIRE (or similar) can be implemented within the REACH Regulation:

An advanced science and technology strategy such as INSPIRE is devised mainly with phase-in or existing substances in mind. However, it should apply equally well to new substances, although final marketing of new substances should be held over until long-term *in vitro* protocols are available. Also new substances may make up a lot of the less hazardous alternatives which the scheme is designed to promote.

A fast-track approach to the development of alternatives to animal testing is crucial in order to protect consumers and the environment. New substances should be subjected to the Phase 1 screening and *in vitro* test batteries (outlined below) so that potentially dangerous substances can be dropped from development early on.

However the marketing of new substances should be held over until the completion of Phase 2 (see below) by which time full profiles from *in vitro* tests should be possible.

The INSPIRE (or similar) strategy is practical, scientifically robust and reasonable. It is important to bear in mind that the animal tests proposed within REACH will not deliver the answers required, so a moratorium until better tests are available is a sensible, precautionary approach.

Computer screening and prioritisation:

Any strategy under REACH must involve a stepwise, inclusive, approach to collecting data, screening, testing, and evaluation which enables all parties involved to contribute.

The immediate and rapid examination of all the chemicals on the market, screened for persistence, bioaccumulation, and toxicity.

A database, managed by the European Chemicals Agency or similar as detailed in the REACH proposal, is created from information currently available. It is important that the central body managing the list includes experts in **bio-informatics** to check the information being submitted.

The central body managing the information should also include experts from outside bodies (see 'Management', later).

Pre-registration

A pre-registration phase follows in which companies should be encouraged to indicate what data they hold on substances – **whether they intend to register the substances or not**. Financial incentives to submit all data held could include fees payable by others wishing to use the data. Data should include use, modes of production, distribution and transport.

During this phase, some of the data would be publicly accessible so that other interested parties, including those from overseas, could contribute to the data packages available.

The database would be publicised to encourage contributions from environmental experts, government and commercial laboratories, government environmental monitoring agencies, academia, and stakeholder groups.

A policy of transparency (where possible) and inclusion of all stakeholders also helps to ensure that data submitted is not only that which indicates the lowest prediction of hazard.

The pre-registration phase would either apply to the 30,000 substances from the European Inventory of Existing Commercial Chemical Substances (EINECS) which are prioritised under REACH, or, **preferably, the whole EINECS list of 100,000** substances.

In this way the problem of data held by those manufacturing or importing at lower tonnage levels not being included would not arise.

This registration phase cannot be extended indefinitely as it will delay the risk assessment process. If limited to 1-2 years it would enable a number of *in vitro* tests to be validated by the end of 2006 and then used to assess the prioritised chemicals.

Screening & Testing:

The UK's Royal Commission on Environmental Pollution has estimated that existing computer screening techniques could be put in place so that all 100,000 EINECS substances could have received an initial screening within 3 years¹.

Persistence, bioaccumulation, bioavailability:

Information on persistence and bioaccumulation already exists for almost all the chemicals on the market, mainly from computer database, analytical, or modelling programmes (as well as

environmental information); these features can also be established with tests using micro-organisms.

Bioavailability (a key determinant of toxicity arising from exposure) and bioaccumulation can be ascertained by means of an already validated test (A8 partition-coefficient), knowledge of molecular size and computer modelling with data derived from *in vitro* tests using micro-organisms. Some of these computer databases are already used by regulatory authorities for screening purposes, so there is a strong precedent for this approach.

Toxicity and ecotoxicity:

Toxicity and ecotoxicity data can be derived from computer analysis and modelling approaches based on chemical structure. For example, the US Environmental Protection Agency's procedure for assessing pre-market notifications uses structure-activity relationships to estimate human acute and chronic toxicity including oncogenicity, mutagenicity, developmental toxicity, neurotoxicity, reproductive toxicity, systemic toxicity, irritability and sensitisation¹. QSARs are used to estimate chronic and acute toxicity values for fish, daphnids and algae.

Culture dish and test-tube methods are an important adjunct to computer models or database systems; it is important to use a bank of the advanced techniques.

Cell lines genetically engineered to express enzymes, either singly or in combination, may be used to identify the principal metabolites of chemicals².

Prioritisation:

Initial screening will enable the sorting of chemicals into high and low priority, whether new or old (EINECS or ELINCS). High priority substances will be divided into two categories:

- (a) persistent, bioaccumulative or both – for immediate phase-out.
- (b) toxic – for further assessment.

Toxic substances are a little less straightforward since for some, the final product is designed to be toxic. The data available will enable further investigation.

It is assumed, working with the development timetable produced by the European Centre for the Validation of Alternative Methods (ECVAM), that a number of *in vitro* tests including those for acute lethal toxicity will be available to produce data for the preliminary risk assessment.

For chemicals already on the market, the preliminary risk assessment will determine whether the substance should be banned immediately, authorised or restricted, or suspended pending review using the *in vitro* tests which will be online by 2010.

New substances which are screened and found to be high priority are not allowed onto the market until they have been the subject of a full risk assessment. This means that there will be a delay in the introduction of new chemicals while the *in vitro* alternatives to the long-term toxicity tests are put in place.

However existing substances which were deemed of low priority in the initial screening will remain on the market until they can be tested.

Industry will need time to remove those substances which have been highlighted as needing substitution in the screening phase (Phase 1).

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However it is not expected that the longer term tests will be available until the end of Phase 2, hence the substitution of safer alternatives is not possible until the end of Phase 2 at least (i.e. 2010).

Although this means there will be a delay of 5 years in getting a replacement for a dangerous chemical onto the market, this is a realistic assessment of how long the process may take. From a consumer perspective the priority must be to remove dangerous substances as quickly as possible.

Importantly, manufacturers will have the option of utilising an existing chemical as opposed to a new one, and as time passes the system will get faster and more efficient.

Development of non-animal methodologies for testing:

No timetables for developing and validating alternatives, along the lines of the Cosmetics Directive for example have been laid down within REACH.

In order that *in vitro* tests could be carried out for the risk assessment it is essential that the budget of ECVAM and the ECVAM Scientific Advisory Committee as well as the European Chemicals Bureau be enlarged to enable them to manage the giant testing programme that is being proposed. It is not realistic to suppose that this can be undertaken without a major acceleration in the development and validation of alternatives.

ECVAM should be consulted on the kinds of resources it would need to carry out its development and validation programme outlined for REACH.

The total cost of REACH to industry has been put by the UK government at between £1,240,735,000 and £4,643,745,914 over 11 years. By comparison, the Fifth Framework Programme allocated €26 million to ECVAM over 4 years, or €6.5 million (£4.25 million) per year. ECVAM's budget should be proportionate to the sums of money envisaged for the animal testing proposals over the same period. **Industry should contribute to an increase in ECVAM's budget.**

It has been suggested that financial constraints are not a factor limiting progress in alternatives; this is hardly credible for at least three reasons:

- Firstly in every other area of research there exists a presumption that more money means more research and greater progress. There is no reason to suppose that this would be different with this one area of scientific research.
- Secondly, the timetable outlined by ECVAM in response to the REACH proposal was not for blue skies research, or for projects in the early phases of development, but for technologies which had already shown considerable promise and progress or needed prevalidation or validation.
- Thirdly it might be argued that above a certain level more money would not do more good simply because there were no more people able to undertake the required research, but it is highly unlikely that the European research community could not meet this challenge.

We would suggest – pending ECVAM's recommendation – an initial budget of something in the region of €145–€290 million (£100–£200 million) should be considered.

Industry will benefit from more economic testing methods and also increased reliability and consistency of data. This should therefore be fed back into better research and development, via further industry support for ECVAM.

Initiative to promote advanced techniques:

We strongly support the recommendation of the UK Government's Royal Commission on Environmental Pollution (RCEP), that the UK Government, together with like-minded Member States, should set up an EU-wide initiative to demonstrate and promulgate the effectiveness of simpler, faster, computer-based methodologies.

The pace at which *in vitro* tests are developed could act as a means for setting deadlines for full risk assessments to the European Chemicals Agency (ECA).

It should be borne in mind that the testing proposals currently within REACH are probably not feasible and so any change to the regulatory timetable based on actual developments and practicability should be welcomed.

The EC has shown with the Cosmetics Directive that industry can respond to a timetable for the development of alternatives to animal tests; a similar strategy should be used for chemicals.

Validation and implementation of technologically advanced, risk-based strategy such as INSPIRE:

Once an alternative method is validated or approved it should be published immediately and a deadline set for introduction. In particular, once alternatives have been established as part of the EU Cosmetics Directive those validated tests which are relevant to REACH should be made a requirement within that programme.

It is essential that the technical annexes of REACH are amended to implement a scientifically and technologically advanced, risk-based *in vitro* testing strategy. To retain animal testing protocols within REACH is at odds with both the aims of the EC White Paper, and Directive 86/609/EEC on animal experiments.

The EU Cosmetics legislation has set a precedent for a strategy which could be utilised for the new regulatory framework for chemicals testing. In view of the fact that the complete range of tests for cosmetics using alternative methodologies must be in place by 2013, with a possible extension of two years, it seems absurd that no equivalent timetable has been written into the draft legislation for chemicals.

Technical problems in respect of toxicokinetics, chronic and reproductive testing are deemed achievable within the next ten years, possibly sooner, so it cannot be the case that the further development of alternative techniques is unachievable within the timescale proposed for the backlog.

Timetables for development and validation of alternatives need to be written into the legislation. These can be agreed with the aid of advisory bodies at Member State and European level.

Where existing alternative methods are adequate to ensure safety, but are not necessarily applicable to all chemical entities, the adoption of these methods at community level must be ensured. At present although such tests could be justified on a case by case basis they still need to be promoted and their use made mandatory.

Current timetable for validation of *in vitro* tests:

In 2002 ECVAM undertook a report of the current status of alternatives in testing as a response to the draft REACH proposal³. Although it would be unfair to hold ECVAM to all of its predictions it serves as a useful model to plan the phasing in of *in vitro* tests. The following areas and schedules were indicated (not including validated alternatives already in place):-

Acute toxicity

Acute toxicity testing for priority setting could already be undertaken using standardised basal cytotoxicity tests. The development of QSARs and other cytotoxicity tests to replace the current rodent tests should be undertaken. It is expected that at the current rate of development alternatives for acute toxicity tests B1, B2 and B3 could be validated by 2006.

Skin irritation

In vitro methods for skin irritation could be used immediately for priority setting, e.g. human skin models like EpiDerm and EPISKIN. QSARs and expert system rulebases need to be developed. Validation by end 2006.

Eye irritation

Poor quality data from *in vivo* tests is the problem here. Provided that better quality reference data is made available a range of validated tests should be available by end 2006, including the fluorescein leakage assay, neutral red uptake assay and neutral red release assay.

Skin sensitisation

Priority setting could begin immediately using QSARs, DEREK, reconstructed epidermis models and dendritic cell cultures. Further fundamental work is required before the validation of *in vitro* systems for skin sensitisation. Validation – end of 2006.

Respiratory sensitisation

Further research and development are needed before an *in vitro* test can be validated. There is no *in vivo* test for this. Prospect for validation of *in vitro* tests – end 2010.

Biokinetics

QSARs predictive of membrane permeability can be used for priority setting immediately, as can *in vitro* methods of skin absorption. Systems to assess passage across the blood-brain barrier need further development. A wide variety of *in vitro* tests are available for identifying metabolic pathways, metabolism mediated toxic effects, metabolic stability and enzyme inhibition. These tests could be used immediately to obtain useful information. Physiologically based biokinetic (PBBK) models integrate physico-chemical, physiological and *in vitro* data. They can be used to determine target organ or system doses. Further development and validation of these systems would be desirable. Validation between 2006 and 2010.

Repeat dose/chronic toxicity

A variety of *in vitro* systems are currently under development, derived mainly from the liver, kidney and brain. Development of these is expected to be long-term with validation from end of 2010 onwards.

Target organ/system toxicity

Attention focuses here on potential effects on the liver, kidneys, nervous and endocrine systems. While a complete toxicological profile derived from the integrated use of alternative tests with complementary endpoints is still at the developmental stage (validation predicted 2006-2010), a number of tests can already yield information which, from the point of view of the requirements of REACH, would be adequate in themselves. Substances of concern might be identified from assessments of barrier function, basal cytotoxicity and energy metabolism alone.

Genotoxicity and carcinogenicity

Screening for potential genotoxicity and carcinogenicity can be performed using *in vitro* systems immediately, and substances could be identified for prioritisation. If the *in vitro* testing is positive for genotoxicity then the substance has the potential to cause cancer and no further testing should be necessary.

The fact that sometimes a mutagen *in vitro* fails to show as a mutagen *in vivo* may relate to deficiencies in the *in vivo* test itself, as the scientific basis of insensitivities of *in vivo* genotoxicity are not known.

In vitro testing for changes in chromosome number may be used to detect the operation of genotoxic mechanisms which only occur *in vivo*. Therefore a battery of *in vitro* tests including computer systems for detecting toxic properties from structure is the way forward.

It is essential that a number of widely-used computer systems are validated for use in genotoxicity screening, e.g. TOPKAT, CASE, COMPACT, DEREK, HazardExpert and ONCOLOGIC as these will improve the speed with which the backlog of testing can be carried out.

Validation of QSARs for genotoxicity has been estimated by ECVAM as a medium term project, and hence should be ready by the end of 2006. Prospects for carcinogenicity testing are considered to be long-term, i.e. end of 2010.

Reproductive toxicity

A number of promising *in vitro* tests are available. In so far as these tests are aimed at the promotion of human health they must focus on the use of human cell and tissue cultures, and evaluate a wide range of toxicological endpoints.

Given the welfare and financial cost of the tests, and the length of time taken to complete them, it is essential that faster, cheaper and more reliable alternative methodologies are used for reproductive toxicity testing.

Since the whole reproductive cycle cannot be modelled in one *in vitro* test it is necessary to study the parts of the system individually and then integrate different endpoints and tests into an overall testing strategy.

However, a battery of non-animal tests has the underlying advantage that the correct species is being studied. There are plans to investigate drug interactions with the human placenta using cell culture models of the placental barrier and in general *in vitro* techniques have shown great promise in providing the tools necessary to elucidate transport and metabolism processes typical of the human placenta⁴.

Research and development in this area may lead to a useful test to form part of the test battery needed to evaluate reproductive toxicity. Semen analysis permits the detection of the effects of chemicals on post-testicular stages in ways that will have a bearing on fertility, viability during embryonic development and health risks to offspring. Different techniques are available for monitoring characteristics of semen and some of these analyses can be automated⁵.

A Lord Dowding Fund for Humane Research project recently established a human testicular tissue bank to facilitate work using human tissue. ECVAM regards this development and validation programme as long term, hence the end of 2010 for results.

Endocrine disruption

The development of non-animal tests for detecting endocrine disruption is in its infancy and no validation date has been estimated by ECVAM. However, it is fair to point out that the reliability and relevance of animal tests is open to question and that there are few, if any, animal tests specifically for EDs. The tests used are merely modifications of existing animal tests.

A number of *in vitro* tests are available using yeasts and MCF-7 breast cancer cell lines. Proliferation in these latter cells is thought to result from oestrogenic substances binding to the oestrogen receptor within the cells. Other *in vitro* assays using human tissues and cell cultures have been developed, including the human adrenocortical carcinoma cell line H295R. The US Environmental Protection Agency is developing structure-activity relationships modelling receptor binding, and these SARs have been extensively studied in Japan. Computer-generated models have been developed to investigate and predict binding to the oestrogen receptor. Genomic analysis using microarrays to screen chemicals for their endocrine disrupting properties is also under way.

It is disappointing that in a new area of toxicology some toxicologists are still travelling down the road of *in vivo* tests using animals, and facing the attendant problems of extrapolating from animal data in the laboratory to the real-life human situation of exposure to small amounts of different chemicals over a long period of time.

In the US a special advisory committee of the Environmental Protection Agency has been set up to advise on the development and validation of assays for ED testing.

The EU should set up a corresponding body to provide research, development and validation advice to build on the success of existing human *in vitro* assays already available and thereby ensure that a rapid and reliable testing regimen is available for this dangerous category of substances⁶. A range of organisations, such as the Lord Dowding Fund, could assist.

Environmental monitoring

Alongside computer-based screening and a fast-track development of *in vitro* methods under ECVAM, the potential for environmental monitoring information must be harnessed.

It is essential that risk assessment is not based on toxicity test data alone. Many of the problems which have arisen with chemicals in the environment have come to light through environmental monitoring¹.

There must be full integration between those responsible for safety assessment of chemicals and those who undertake environmental assessment. Authorities tend to focus only on animal test results, when they are not integrated with other sources of information.

Environmental monitoring does not require the performance of live animal tests. Natural deaths in the environment can be used for screening for important biomarkers of hazard, and other methods are available such as population monitoring, including human and environmental epidemiological monitoring, plant-based bioassay techniques and *in vivo* tests using genetically modified bacteria which detect the presence of toxins.

The UK's RCEP has suggested the introduction of a scheme whereby anyone can submit information regarding environmental hazard or accident; this would be modelled on the 'yellow card' scheme used by doctors to communicate information on adverse drug reactions. Even casual observations by members of the public could be used to set in motion the investigation of unusual biological effects, especially important in cases where existing toxicity tests may be of limited value, e.g. in the case of endocrine disruption. **We support this suggestion.**

RCEP recommends that the integration of the results of certain types of environmental monitoring into the chemicals assessment and management programme is perfectly possible and would result in a much more effective system without a major increase in costs.

Environmental monitoring information should be added to the database and inform prioritisation and risk assessment of substances. The process would be ongoing and would act as a potential trigger for reassessment of substances. In any case, chemicals found in unexpected environmental compartments or at unexpected concentrations, or associated with unusual biological phenomena should be selected for further investigation¹.

Management and Review, transparency, inclusion:

As mentioned earlier, the Management Board of the European Chemicals Agency should comprise at least one member from the animal protection movement, one from the non-animal research funding sector, and one from the environmental movement. The Board should be required to hold regular meetings with non-animal research funding bodies, to discuss developments in the field. Bodies such as the Lord Dowding Fund should be invited to contribute.

The Commission should undertake a review of progress perhaps every three years, again in the way that has been stipulated under the Cosmetics Directive, and determine priorities in consultation with Commission agencies and Member States.

A review will serve not only to help keep technical progress on track but also to indicate those tests which will need to be undertaken to fill data gaps for the prioritised substances.

This will also serve as the baseline for working out deadlines for completion of data sets and risk assessments under a revised REACH, incorporating the INSPIRE approach.

Timetable:

The following timetable is provisional and will depend in part on how long it takes to complete the development and validation of suitable alternatives. In practice many of the procedures in different phases will overlap as they will be ongoing. Also it is not possible to give accurate estimates of how many substances of concern will be identified and hence how long the programme of work for each phase will finally take. Although failure to hit deadlines may be inevitable it is essential that there be mechanisms in place to assure the necessary commitment to progress. There must be sufficient flexibility in the timetable to make possible the phasing out of hazardous substances by industry.

Where appropriate the following proposal assumes the use of certain agencies and procedures outlined in the REACH Regulation:-

Phase 1 (Years 1-3):

- Setting list and entry onto database; computer screening of chemicals for persistence, bioaccumulative potential and toxicity – either all EINECS or 30,000 REACH priority substances.
- Further prioritisation based on likely exposure and, if necessary use of existing *in vitro* tests such as those for acute lethal toxicity (NRU), skin irritation (EpiDerm and EPISKIN), skin sensitisation, biokinetics, target organ/system toxicity and genotoxicity to facilitate categorisation into higher and lower concern groups.
- Substantial increase in the ECVAM budget; revision of ECVAM timetable for *in vitro* development and validation. ECVAM to establish work schedules with industry.
- Early use of validated alternatives to contribute to preliminary risk assessments of substances identified as a priority. Validated alternatives to acute lethal toxicity, skin irritation and eye irritation should be available by 2006 (end of year 2). Risk assessments transmitted to regulatory bodies for early evaluation of hazard. Such substances will either be banned, authorised, restricted, or suspended or allowed on the market pending further validation of tests.
- Extended pre-registration period to determine data availability.
- Environmental monitoring (ongoing) – ban on synthetic substances found at elevated levels in biological fluids, prioritisation of others linked to environmental anomalies.
- Phasing out of persistent and bioaccumulative substances
- Green chemistry initiatives (ongoing)
- Review of progress in alternatives; announcement of tests ready to fill data gaps. Deadlines drawn up for fulfilling certain data requirements.
- Beginning of collection of toxicity data for new substances to replace those identified as hazardous by the screening programme

Phase 2 (Years 4-6):

- Application of validated *in vitro* tests to priority substances identified in screening, including those resulting from Cosmetics Directive initiative (projected validation of *in vitro* tests for reproductive toxicity, carcinogenicity, chronic toxicity and respiratory sensitisation = 2010).
- Provision of time for industry to carry out substitution; including further *in vitro* testing for substitutes for hazards identified from Phase 1 and 2.
- Further data from environmental monitoring added to database leading either to restriction, substitution etc.
- Further development by ECVAM of alternatives
- Review of progress in alternatives; announcement of tests ready to fill data gaps for prioritised substances. Deadlines drawn up for fulfilling data requirements.

Phase 3 (Years 6-9):

- Further development and validation of *in vitro* tests
- Continuation of application of newly validated *in vitro* tests, including those from Cosmetics Directive initiative, to prioritised substances.
- Time for industry to key in alternatives arising from testing in Phase 2.
- Beginning of application of whole new sets of *in vitro* test batteries to substances which have not been prioritised and subsequent risk assessment
- Further data from environmental monitoring
- Review of technical progress and announcement of which tests are now ready to fill data gaps for prioritised and non-prioritised substances. Deadlines drawn up for fulfilling certain data requirements.
- Completion of testing for substitutes for hazardous substances identified in Phase 1 and 2.

Phase 4 (Years 9- onwards):

- Further development of *in vitro* tests (ongoing)
- Further data from environmental monitoring
- Risk assessments using *in vitro* testing protocols applied to non-prioritised and newly prioritised substances.
- Review of technical progress and implementation of further strategies for dealing with existing chemicals. Deadlines drawn up for testing and risk assessment (in 3 year periods if found to be satisfactory)
- Time for industry to key in alternative products and processes for substitution outlined in Phase 3

Summary of advantages of a technologically advanced, risk-based strategy:

The advantages of a scientifically and technologically advanced, risk-based strategy over the current REACH proposals are clear:-

- It avoids the massive animal testing programme advocated by REACH, saving millions of animals, thus fulfilling the objectives of both REACH and **Directive 86/609/EEC** with respect to avoiding the use of animals in research.
- It is based on a testing system which is relevant to the species being studied, scientifically and technologically advanced, drawing on a multi-disciplinary and intelligent approach, as well as capture of data from a wider range of sources.
- It is based on a proper risk assessment, including bioavailability rather than a crude measure such as tonnage of production, thus allowing a quicker investigation of substances of concern.
- Cheaper, faster and more reliable *in vitro* testing methods will give the smaller and speciality enterprises within the European chemicals sector more freedom to innovate.
- Industry as a whole will benefit from losing the burden of a costly, time-consuming and cumbersome animal testing programme.
- Less animal testing will mean more funds are released for environmental monitoring, green chemistry initiatives, and substitution of hazardous chemicals.
- Unlike REACH the testing programme is flexible, achievable and sustainable.
- INSPIRE or a similar strategy does not require a huge amount of co-operation between companies, and if companies do not share data this is not critical to the workability of the system.
- INSPIRE (or similar) augments the sources of information on which risk assessment is based, making it more reliable in respect of ecotoxicology.
- INSPIRE (or similar) does not require penalties for non-compliance, with all the potential difficulties which that entails.

This strategy of focus upon scientifically and technologically advanced research and testing techniques delivers the objectives of REACH.

1 Royal Commission on Environmental Pollution. Chemicals in Products. HMSO, UK. June 2003.

2 Combes, R et al. ATLA 2003; 31: 7-19

3 ATLA 2002; 30 Suppl 1.

4 Audus, KL. Controlling drug delivery across the placenta. Eur J Pharm Sci 1999; 8(3): 161-165

5 Worth, A and Balls, M. (Non-animal) Methods for Chemicals Testing: Current Status and Future Prospects. ATLA 2002; 30 Suppl 1: 95-102

6 Worth, A and Balls, M. (Non-animal) Methods for Chemicals Testing: Current Status and Future Prospects. ATLA 2002; 30 Suppl 1: 103-113

7 Commission of the European Communities. White Paper. Strategy for a Future Chemicals Policy. Brussels. 27/2/2002. COM (2001) 88 Final.

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