

Chapter 1: Background

The White Paper on a Strategy for a Future Chemicals Policy

The legal requirements for the testing of chemicals in the European Union (EU) are laid down by four main pieces of legislation:

1. the “Dangerous Substances Directive” (*Council Directive 67/548/EEC* [1]) and its subsequent amendments and adaptations to technical progress;
2. the “Dangerous Preparations Directive” (*Council Directive 88/379/EEC* [2]) and its subsequent amendments and adaptations to technical progress;
3. *Council Regulation (EEC) No. 793/93* on the evaluation and control of risks of existing substances (3); and
4. the “Limitations Directive” (*Council Directive 76/769/EEC* [4]) and its subsequent amendments and adaptations to technical progress.

The current system of EU chemicals legislation distinguishes between “existing substances”, i.e. chemicals that were declared to be on the market on or before 18 September 1981, and “new substances”, i.e. chemicals that have been placed on the market since that date. A total of 100,195 existing chemicals are listed in the European Inventory of Existing Commercial Chemical Substances (EINECS), and approximately 3000 new substances have been notified since 1981.

It is now recognised that there are a number of weaknesses in the current system:

1. new and existing chemicals are not subject to the same testing requirements, which means that there is a lack of knowledge about the potential danger represented by many existing substances, which represent about 99% of the total volume of chemicals on the market;
2. the current process of risk assessment is much too slow, with only a handful of existing chemicals being assessed each year; and
3. resources are concentrated too much on the assessment of new chemicals, which make up only about 1% of the total volume of substances on the market.

With a view to overcoming the weaknesses in the current system, on 13 February 2001, the European Commission adopted a *White Paper on a Strategy for a Future Chemicals Policy* (5). The main objective of the new policy is to ensure a high level of protection for human health and the environment, while ensuring the efficient functioning of the internal market and protecting the competitiveness of the EU chemical industry.

The White Paper proposes to harmonise the testing requirements for new and existing substances, by introducing a new system for the Registration, Evaluation and Authorisation of new and existing chemical substances, known as the REACH system. Registration will require producers, importers and, where necessary, downstream users, to deposit information with a central authority (possibly, the European Chemicals Bureau, a unit of the European Commission’s Joint Research Centre), having conducted such additional testing as may have been necessary. The evaluation of these data will be managed by Competent Authorities in the Member States, and may lead to further requirements for testing substances produced in quantities of more than 1 tonne/enterprise/year. Authorisation will apply to chemicals of very high concern, including carcinogenic, mutagenic and reprotoxic (CMR) substances (categories 1 and 2), and to so-called “persistent organic pollutants” (POPs), irrespective of their production volumes. It is also proposed that a system of restrictions will apply to substances of concern, which might include persistent bioaccumulative and toxic substances (PBTs), and very persistent and very bioaccumulative substances (VPVBs).

It is estimated that implementation of the new chemicals policy will result in the need for the further assessment of up to 30,100 existing chemicals, which are currently marketed in volumes greater than 1 tonne per year (tpa), and for which essential human health and ecotoxicological data are lacking. The proposed schedule for registration depends on the production/importation volume of the chemical (Table 1.1).

The amount of testing required will be triggered partly by the production/importation volume (Table 1.2). For example, it is further proposed that chemicals with volumes in the range 1–10 tpa should be tested with *in vitro* methods *alone*, which means that a set of appropriate alternative methods should be available to permit the registration of such substances. It should also be noted that chemicals produced/imported in amounts higher than 10 tpa are not necessarily excluded from *in vitro* testing. In addition, the testing of chemicals of partic-

ular concern, such as CMRs and POPs, may be required, even if they are marketed in volumes of less than 1 tpa.

Follow-up to the White Paper

The White Paper was discussed by the Environment Council on 7 June 2001 (6), when it was concluded, *inter alia*, that:

“Animal testing should be limited to the level necessary to deliver the objectives of the strategy, including a high level of protection for human health and the environment. Industry should make all existing data available to avoid duplication of testing. Mechanisms are needed to ensure that unnecessary testing requirements are avoided. Adequate resources must be provided for research, development and validation of globally accepted test guidelines for alternative *in vitro* test methods, so that work can be accelerated at all levels. Activities under the new Framework Programme for Research should consider these requirements among its priorities. In addition to promoting this issue in ECVAM (European Centre for the Validation of Alternative Methods), the Community should play a more active role in the OECD, to encourage wider adoption of validated, alternative, non-animal testing methods.”
(*Council Conclusion 23*)

The White Paper has also been discussed in the European Parliament (EP) and in the Economic and Social Committee (ESC). On 15 November, a plenary session of the EP adopted a report on the White Paper produced by Mrs Schörling MEP. The Schörling report (7) sets out a whole series of rules that would restrict the number of animal tests and foster the development of alternative methods. On 17 October 2001, the ESC adopted an opinion on the White Paper, in which it endorsed the commitment to promote non-animal testing (8).

Table 1.1: The proposed schedule for the registration of 30,100 existing substances (6)

Number of substances	Volume (tonnes per annum)	Deadline for registration
2600	>1000	end of 2005
2900	100–1000	end of 2008
4600	10–100	end of 2012
20,000	1–10	end of 2012

In order to prepare legislative proposals for the implementation of the White Paper, the Commission has established eight Working Groups, to obtain scientific and technical advice from experts nominated by the EU Member States, industry organisations and non-governmental organisations. It is expected that a legislative proposal, based on the advice of the Commission Working Groups, will be drafted by the summer of 2002, after which it will be considered for adoption by the Council and the Parliament, according to the co-decision procedure.

Directive 86/609/EEC and ECVAM

In 1986, the Council of Ministers adopted *Directive 86/609/EEC* on the Protection of Laboratory Animals for Experimental and Other Scientific Purposes (9). Article 23 of *Directive 86/609/EEC* states that:

“The Commission and Member States should encourage research into the development and validation of alternative techniques, which could provide the same level of information as that obtained in experiments using animals, but which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field.”

In October 1991, the European Commission responded to this article by means of a Communication to the Council and the Parliament (9), which established ECVAM, as a unit of the Joint Research Centre. The mission of ECVAM is to play a leading role at the European level in the independent evaluation of the relevance and reliability of tests for specific purposes, through research on advanced methods and new test development and validation.

Alternative Methods and Their Application

In the context of laboratory animal use, *alternatives* include “all procedures which can completely replace the need for animal experiments, reduce the number of animals required, or diminish the amount of distress or pain suffered by animals in meeting the essential needs of man and other animals” (11).

This definition embodies the Three Rs concept proposed by Russell & Burch in *The Principles of Humane Experimental Technique* (12). The laws of many countries and *Directive 86/609/EEC* of the European Union now specifically require that

Table 1.2: The dependence of testing requirements on tonnage (6)

Volume (tonnes per annum)	Testing required
1–10	“Testing should generally be limited to <i>in vitro</i> methods”
10–100	Base-set testing
100–1000	Base-set testing + Level 1 testing, i.e. “substance-tailored testing for long-term effects”
>1000	Base-set testing + Level 2 testing, i.e. “additional substance-tailored testing for long-term effects”

Table 1.3: Membership of the ECVAM Working Group on Chemicals

Member	Contact details
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replacement alternatives, reduction alternatives and refinement alternatives should be used wherever and whenever possible in biomedical research, testing and education.

The certainty that some additional testing will be necessary in relation to the future Chemicals Policy, albeit on a scale which cannot be predicted precisely at present, will necessitate the vigorous and dedicated application of all Three Rs, so that any suffering caused to the animals that will have to be used will truly be unavoidable “in meeting the essential needs of man and other animals”.

This ECVAM report will primarily focus on replacement alternative tests and testing strategies, but there is a no less urgent need for attention to be focused on the implications of the Future Chemicals Policy in terms of the other two Rs.

There is undoubtedly scope for reducing the numbers of animals required through a reconsideration of group sizes and of test designs (13). The statistical method and experimental design adopted for a given study should maximise the scientific output from the use of a given number of animals. Furthermore, the extent and frequency of unavoidable animal suffering could undoubtedly be decreased through the development and use of the earliest and most-humane endpoints that meet the scientific objectives (14). This will be particularly important for the assessment of endpoints for which non-animal replacements are unlikely to be available in the near future (for example, in chronic toxicity testing).

In addition, there should be an authoritative and independent review of the relevance and reliability of all the animal-test procedures that will eventu-

Table 1.4: Base-set testing requirements for human health endpoints

Endpoint	EU test methods
Acute toxicity	[B.1: acute toxicity (oral) — DELETED ON 25/1/01] B.1bis: acute toxicity (oral) fixed dose method B.1tris: acute toxicity (oral) — acute toxic class method B.2: acute toxicity (inhalation) B.3: acute toxicity (dermal)
Irritation	B.4: acute toxicity (skin irritation) B.5: acute toxicity (eye irritation)
Corrosivity	B.40: skin corrosion
Skin and respiratory sensitisation	B.6: skin sensitisation No Annex V method for respiratory sensitisation
Repeated dose toxicity	B.7: repeated dose (28 days) toxicity (oral) B.8: repeated dose (28 days) toxicity (inhalation) B.9: repeated dose (28 days) toxicity (dermal)
Mutagenicity and genotoxicity	B.10: mutagenicity (<i>in vitro</i> mammalian chromosome aberration test) B.11: mutagenicity (<i>in vivo</i> mammalian bone-marrow chromosome aberration test) B.12: mutagenicity mammalian erythrocyte micronucleus test B.13/14: mutagenicity — reverse mutation test using bacteria B.15: gene mutation — <i>Saccharomyces cerevisiae</i> B.16: mitotic recombination — <i>Saccharomyces cerevisiae</i> B.17: mutagenicity — <i>in vitro</i> mammalian cell gene mutation test B.18: DNA damage and repair — unscheduled DNA synthesis — mammalian cells <i>in vitro</i> B.19: sister chromatid exchange assay <i>in vitro</i> B.20: sex-linked recessive lethal test in <i>Drosophila melanogaster</i> B.21: <i>in vitro</i> mammalian cell transformation test B.22: rodent dominant lethal test B.23: mammalian spermatogonial chromosome aberration test B.24: mouse spot test B.25: mouse heritable translocation B.39: unscheduled DNA synthesis (UDS) test with mammalian liver cells <i>in vivo</i>

Based on Annexes VII A, B and C of Directive 67/548/EEC.

ally be required for chemicals for which further testing must be conducted, in compliance with the REACH system.

Furthermore, the Three Rs should not be seen as separate entities, but as complementary elements in a common approach to humane science. For example, it is often more appropriate to think, not only of replacements *per se*, but of *non-animal tests and testing strategies*, especially where their use can lead, not to total replacement, but to a reduction in numbers of animals required, through the better prediction of starting doses or the use of fewer treatment groups. Similarly, information obtained in a non-animal test can frequently eliminate the risk that severe effects will be caused to animals necessarily used (for example, to confirm a negative result or a low-level effect).

The application of an integrated approach to essential testing should involve the simultaneous consideration of all the Three Rs, as well as the integrated (and non-competitive) use of the variety of non-animal tests and non-animal test data that are, or will increasingly become, available. These include:

1. Maximising the use of existing information, including the reasons for producing a chemical and its uses, as well as knowledge of its toxic hazard potential.
2. The use of data concerning the physicochemical properties of chemicals (for example, stability, solubility, pH, octanol–water partition coefficient, protein binding).
3. Predictions based on structure–activity relationships, including qualitative and quantitative

mathematical models, and the use of read-across data from related chemicals.

4. The biokinetic modelling of physiological, pharmacological and toxicological processes.
5. Experiments on lower organisms not classed as “protected animals” (bacteria, fungi, plants, invertebrate animals).
6. Studies on vertebrates at early stages of development (before they become “protected animals”).
7. Studies on *in vitro* systems of various kinds (including whole perfused organs, tissue slices, cell, tissue and organotypic cultures, and sub-cellular fractions).
8. Human studies (including estimations of occupational and environmental exposure, epidemiological investigations, post-marketing surveillance for medicines, cosmetics and household and agricultural products, and the ethical and properly controlled use of human volunteers).

The Role of ECVAM in the Formulation of the EU Chemicals Policy

ECVAM is playing an advisory role in the formulation of the EU Chemicals Policy, by contributing to the inter-service discussions within the Commission that aim to elaborate the details of the new policy. To help ECVAM in this role, ECVAM established a Working Group on Chemicals

Table 1.5: Level 1 testing requirements for human health endpoints

Endpoint	EU test methods
Sub-chronic and/or chronic toxicity	B.26: sub-chronic oral toxicity test: 90-day repeated oral dose study using rodent species B.27: sub-chronic oral toxicity test: 90-day repeated oral dose study using non-rodent species B.28: sub-chronic dermal toxicity test: 90-day repeated dermal dose study using rodent species B.29: sub-chronic inhalation toxicity test: 90-day repeated inhalation dose study using rodent species B.30: chronic toxicity test
Developmental toxicity	B.31: teratogenicity test — rodent and non-rodent
Fertility study	B.34: one-generation reproduction toxicity test B.35: two-generation reproduction toxicity test
Additional mutagenicity studies	
Toxicokinetics	B.36: toxicokinetics

Based on Annex VIII of Directive 67/548/EEC.

Table 1.6: Level 2 testing requirements for human health endpoints

Endpoint	EU test methods
Chronic toxicity	B.30: chronic toxicity test
Carcinogenicity	B.21: <i>in vitro</i> mammalian cell transformation test B.32: carcinogenicity test B.33: combined chronic toxicity/carcinogenicity test
Developmental toxicity	Using species not used in Level 1 study
Developmental toxicity	For peri-natal and post-natal effects
Fertility study	Extended B.35: three-generation reproduction toxicity test
Additional pharmacokinetic studies	To cover, for example, biotransformation
Additional organ or system toxicity	B.7: includes neurotoxicity and immunotoxicity B.37: delayed neurotoxicity of organophosphorus substances following acute exposure B.38: delayed neurotoxicity of organophosphorus substances 28-day repeated dose study

Based on Annex VIII of Directive 67/548/EEC.

(referred to hereafter as the Working Group) in July 2001. The remit given to the Working Group was to propose, by the end of 2001, a strategy on alternative (non-animal) methods in relation to the emerging Chemicals Policy. Details of the membership of the Working Group are given in Table 1.3.

At its first meeting on 24–25 July 2001, the Working Group decided to focus its discussions and recommendations on the use of alternative methods for the hazard assessment of toxicological endpoints for human health (rather than ecotoxicological endpoints), with particular emphasis on the toxicological endpoints currently required in the EU for chemicals testing, i.e. the endpoints defined in Annexes VII and VIII of *Directive 67/548/EEC* (Tables 1.4–1.6).

The Working Group decided to produce a detailed review of the current status of alternative methods for chemicals testing, which would also include proposals for the strategic use of alternative tests, and a three-stage action plan, containing proposals reflecting the short-, medium- and long-term prospects for the development and validation of alternative tests (Table 1.7).

To help with the production of the review document, the Working Group recommended that nine ECVAM Focus Groups should be established, which would focus their efforts on nine areas corresponding to the different toxicological endpoints.

The membership of the ECVAM Focus Groups on Toxicological Endpoints is given in Table 1.8. Additional experts were also consulted on specific issues, when necessary.

The Working Group had further meetings, on 12 October 2001 and 21–22 January 2002, to discuss the progress being made with the ECVAM review document, and to exchange ideas for the strategic use of alternative tests. On 23 January, a draft version of the document was discussed at ECVAM with a number of experts and stakeholders who had not previously been involved in its production (Table 1.9).

Table 1.7: A proposed time-frame for the development and validation of alternative methods for chemicals testing

Priority	Time-frame for completion of activities
Short-term	2003
Medium-term	2006
Long-term	2010

Adherence to this time-frame will depend on many factors, including the availability of human and financial resources.

Besides the White Paper (5), the Working Group based its discussions and recommendations on a number of other documents, including:

1. the conclusions on the chemicals policy reached by the European Environment Council, in its meeting of 7 June 2001 (6);
2. a document published by the British Union for the Abolition of Vivisection (15), containing a proposal for a non-animal testing strategy; documents published by the Medical Research Council's Institute for Environment and Health, UK, consisting of assessments of: a) the implications of the future chemicals policy, in terms of financial cost and animal use (16); and b) the feasibility of replacing current regulatory *in vivo* tests with *in vitro* tests (17); and
3. a document about the TestSmart Program in the USA (18). Further information on this programme is available from: <http://caat.jhsph.edu/programs/workshops/testsmart/hpv-intro.htm>.

The Role of ECVAM in the Implementation of the EU Chemicals Policy

ECVAM will also play a role in the implementation of the future chemicals policy. In Section 3.2 of the White Paper, it is stated that:

“One of the major tasks of the European Centre for the Validation of Alternative Methods (ECVAM) of the Joint Research Centre of the Commission is to validate alternative methods that reduce, refine or replace animal experiments . . .

“ECVAM's central role will be maintained, and it is expected that the development of alternative methods will be accelerated. Further research will be carried out both at Community and national level, to develop and validate novel testing strategies involving fewer or no animals, while enhancing the relevant information that can be obtained from testing without simultaneously increasing the number of animals involved.”

Table 1.8: Membership of the ECVAM Focus Groups on Toxicological Endpoints

ECVAM Working Group	ECVAM coordinator	External partners
Acute systemic toxicity	Silvia Casati	Manfred Liebsch (ZEBET) Richard Clothier (FRAME)
Acute local toxicity Skin irritation/corrosion Eye irritation/corrosion	Andrew Worth Valérie Zuang	Julia Fentem (Unilever) Philip Botham (Syngenta)
Sensitisation (skin and respiratory)	Valérie Zuang	David Basketter (Unilever) Martin Barratt (Marlin Consultancy)
Repeat-dose toxicity	Pilar Prieto	Walter Pfaller (University of Innsbruck)
Genotoxicity and carcinogenicity	Enrico Sabbioni	Robert Combes (FRAME)
Reproductive toxicity	Susanne Bremer	Horst Spielmann (ZEBET)
Neurotoxicity	Sandra Coecke	Erik Walum (Biovitrum)
Toxicokinetics — absorption and distribution <i>In vitro</i> barrier function tests (blood–brain barrier, gastrointestinal tract, kidney)	Pilar Prieto	Per Garberg (Biovitrum)
Computer modelling of absorption and distribution	Andrew Worth	Bas Blaauboer (RITOX) Mark Cronin (Liverpool John Moores University)
Toxicokinetics — metabolism	Sandra Coecke	Johannes Doehmer (GenPharmTox BioTech AG)

Table 1.9: Additional participants in the ECVAM stakeholders meeting held on 23 January 2002

Bernward Garthoff	Bayer AG, Germany
Paul Harrison	Institute for Environment and Health, Leicester University, UK
Ian Indans	Health & Safety Executive, UK
Michael Jackson	Consultant, Nuthampstead, Herts, UK
Gill Langley	Dr Hadwen Trust, UK
Beatrice Lucaroni	DG RTD/E.4, European Commission
Barry Phillips	Royal Society for the Prevention of Cruelty to Animals, UK
Juan Riego-Sintes	ECB, IHCP, JRC, European Commission
Arturo Sanabria	DG SANCO/C.2, European Commission
Regina Schumann	DG ENTR/F.3, European Commission
Kees van Leeuwen	Director of IHCP, JRC, European Commission
Ursula Sauer	Akademie für Tierschutz, Neubiberg, Germany

Excluding members of ECVAM staff and the Working Group on Chemicals.

An additional role for ECVAM is referred to in Section 6 of the White Paper, which outlines the provisions for the testing and evaluation of existing substances, and envisages the establishment of an advisory task force of experts from the Member States before the new legislation is implemented. These experts will be assigned various responsibilities, including (for substances exceeding 1000 tpa) the proposal of additional testing programmes. It is clearly stated that these proposals should be made “in cooperation with ECVAM”.

The future role of ECVAM is also referred to in the conclusions of the European Environment Council on the chemicals policy (6), reached during its 2355th session, held on 7 June 2001. In paragraph 23 of the Council conclusions, it is stated that:

“Adequate resources must be provided for research, development and validation of globally accepted test guidelines for alternative in vitro test methods, so that work can be accelerated at all levels. Activities under the new Framework Programme for Research should consider these requirements among its priorities. In addition to promoting this issue in ECVAM (European Centre for the Validation of Alternative Methods), the Community should play a more active role in the OECD, to encourage wider adoption of validated, alternative, non-animal testing methods.”

Besides its role in the validation of alternative methods, and in promoting the regulatory acceptance of alternative methods (19), ECVAM also plays an important role as an information centre and in providing a forum for discussion. For the dissemination of information, ECVAM is developing a Scientific Information Service (ECVAM SIS), which

is available on the Internet (<http://ecvam-sis.jrc.it>). It contains comprehensive information about validation studies, including the experimental protocols of validated tests and tests under development, and test data generated in validation and other studies.

ECVAM also provides a forum for scientific discussion through its workshops, at which experts from academia, industry and other communities discuss the state of the art in a particular field, and make recommendations for further progress. To date, the reports of 46 ECVAM workshops have been published (Appendix 1.1).

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Appendix 1.1: List of ECVAM workshops

No.	Title	Venue/date	Publication details
1	The Practical Applicability of Hepatocyte Cultures in Routine Testing	Angera, Italy 19–23 October 1993	Blaauboer <i>et al.</i> (1994) <i>ATLA</i> 22 , 231–241
2	<i>In Vitro</i> Phototoxicity Testing	Angera, Italy 13–17 December 1993	Spielmann <i>et al.</i> (1994) <i>ATLA</i> 22 , 314–348
3	<i>In Vitro</i> Neurotoxicity Testing	Angera, Italy 7–11 February 1994	Atterwill <i>et al.</i> (1994) <i>ATLA</i> 22 , 350–362
4	Alternatives to Animal Testing in the Quality Control of Immunobiologicals: Current Status and Future Prospects	Utrecht, The Netherlands 16–17 April 1994	Hendriksen <i>et al.</i> (1994) <i>ATLA</i> 22 , 420–434
5	Practical Aspects of the Validation of Toxicity Test Procedures	Amden, Switzerland 24–28 January 1994	Balls <i>et al.</i> (1995) <i>ATLA</i> 23 , 129–147
6	A Prevalidation Study on <i>In Vitro</i> Skin Corrosivity Testing	Angera, Italy 12–14 January 1994	Botham <i>et al.</i> (1995) <i>ATLA</i> 23 , 219–255
7	Development and Validation of Non-animal Tests and Testing Strategies: the Identification of a Coordinated Response to the Challenge and the Opportunity Presented by the Sixth Amendment to the Cosmetics Directive (76/768/EEC)	Angera, Italy 11–13 April 1994	Balls <i>et al.</i> (1995) <i>ATLA</i> 23 , 398–409
8	The Integrated Use of Alternative Approaches for Predicting Toxic Hazard	Angera, Italy 23–27 January 1995	Barratt <i>et al.</i> (1995) <i>ATLA</i> 23 , 410–429
9	Safety and Efficacy Testing of Hormones and Related Products	Düsseldorf, Germany 25–27 November 1994	Garthoff <i>et al.</i> (1995) <i>ATLA</i> 23 , 699–712
10	Nephrotoxicity Testing <i>In Vitro</i>	Angera, Italy 16–20 May 1994	Hawksworth <i>et al.</i> (1995) <i>ATLA</i> 23 , 713–727
11	The Three Rs: The Way Forward	Sheringham, UK 30 May–3 June 1996	Balls <i>et al.</i> (1995) <i>ATLA</i> 23 , 838–866
12	Screening Chemicals for Reproductive Toxicity: the Current Alternatives	Angera, Italy 22–26 February 1994	Brown <i>et al.</i> (1995) <i>ATLA</i> 23 , 868–882
13	Methods for Assessing Percutaneous Absorption	Angera, Italy 30 May–3 June 1994	Howes <i>et al.</i> (1996) <i>ATLA</i> 24 , 81–106
14	The Use of <i>In Vitro</i> Systems for Evaluating Haematotoxicity	Angera, Italy 29 May–2 June 1995	Gribaldo <i>et al.</i> (1996) <i>ATLA</i> 24 , 211–231
15	The Use of Biokinetics and <i>In Vitro</i> Methods in Toxicological Risk Evaluation	Utrecht, The Netherlands 21–23 March 1995	Blaauboer <i>et al.</i> (1996) <i>ATLA</i> 24 , 473–497
16	Acute Toxicity Testing <i>In Vitro</i> and the Classification and Labelling of Chemicals	Angera, Italy 18–22 April 1994	Seibert <i>et al.</i> (1996) <i>ATLA</i> 24 , 499–510
17	Alternatives to the Animal Testing of Medical Devices	Copenhagen, Denmark 24–26 November 1995	Svendson <i>et al.</i> (1996) <i>ATLA</i> 24 , 659–669

Appendix 1.1: continued

No.	Title	Venue/date	Publication details
18	<i>In Vitro</i> Tests for Respiratory Toxicity	Angera, Italy 14–18 November 1994	Lambré <i>et al.</i> (1996) <i>ATLA</i> 24 , 671–681
19	Alternative Methods for Skin Sensitisation Testing	Angera, Italy 24–28 April 1995	de Silva <i>et al.</i> (1996) <i>ATLA</i> 24 , 683–705
20	The Use of Tissue Slices for Pharmacotoxicology Studies	Angera, Italy 27–31 May 1996	Bach <i>et al.</i> (1996) <i>ATLA</i> 24 , 893–923
21	The Production of Avian (Egg Yolk) Antibodies: IgY	Berlin, Germany 22–24 March 1996	Schade <i>et al.</i> (1996) <i>ATLA</i> 24 , 925–934
22	Pharmacokinetics in Early Drug Research	Bath, UK 27–29 March 1996	Leahy <i>et al.</i> (1997) <i>ATLA</i> 25 , 17–31
23	Monoclonal Antibody Production	Angera, Italy 19–22 November 1996	Marx <i>et al.</i> (1997) <i>ATLA</i> 25 , 121–137
24	The Development and Validation of Expert Systems for Predicting Toxicity	Angera, Italy 1–4 October 1996	Dearden <i>et al.</i> (1997) <i>ATLA</i> 25 , 223–252
25	Current Status and Future Developments of Databases on Alternative Methods	Neubiberg, Germany 12–15 September 1996	Janusch <i>et al.</i> (1997) <i>ATLA</i> 25 , 411–422
26	Genetically Engineered Cell Lines: Characterisation and Applications in Toxicity Testing	Angera, Italy 26–27 February 1996	Wiebel <i>et al.</i> (1997) <i>ATLA</i> 25 , 625–639
27	Issues Relating to the Release of Proprietary Information and Data for Use in the Validation of Alternative Methods	Munich, Germany 9–11 May 1997	Todd <i>et al.</i> (1998) <i>ATLA</i> 26 , 13–20
28	The Use of Transgenic Animals in the European Union	Southwell, UK 7–11 April 1997	Mephram <i>et al.</i> (1998) <i>ATLA</i> 26 , 21–43
29	Reducing the Use of Laboratory Animals in Biomedical Research: Problems and Possible Solutions	Southwell, UK 12–15 January 1998	Festing <i>et al.</i> (1998) <i>ATLA</i> 26 , 283–301
30	Non-animal Tests for Evaluating the Toxicity of Solid Xenobiotics	Angera, Italy 28–31 October 1997	Fubini <i>et al.</i> (1998) <i>ATLA</i> 26 , 579–617
31	Validation of Alternative Methods for the Potency Testing of Vaccines	Angera, Italy 14–16 November 1997	Hendriksen <i>et al.</i> (1998) <i>ATLA</i> 26 , 747–761
32	The Availability of Human Tissue for Biomedical Research	Barnsdale, Rutland, UK 18–22 May 1998	Anderson <i>et al.</i> (1998) <i>ATLA</i> 26 , 763–777
33	Alternatives to the Use of Animals in Higher Education	Crete, Greece 8–10 May 1998	Van der Valk <i>et al.</i> (1998) <i>ATLA</i> 27 , 39–52
34	Eye Irritation Testing: The Way Forward	Egham, UK 15–17 June 1998	Balls <i>et al.</i> (1999) <i>ATLA</i> 27 , 53–77
35	The Production of Polyclonal Antibodies in Laboratory Animals	Utrecht, The Netherlands 20–22 March 1998	Leenaars <i>et al.</i> (1999) <i>ATLA</i> 27 , 79–102.

Appendix 1.1: continued

No.	Title	Venue/date	Publication details
36	The Potential Use of Non-invasive Methods in the Safety Assessment of Cosmetic Products	Brussels, Belgium 10–12 March 1998	Rogiers <i>et al.</i> (1999) <i>ATLA</i> 27 , 515–537
37	The Principles of Good Laboratory Practice: Application to <i>In Vitro</i> Toxicology Studies	Angera, Italy 6–9 December 1998	Cooper-Hannan <i>et al.</i> (1999) <i>ATLA</i> 27 , 539–577
38	The Use of Human Keratinocytes and Human Skin Models for Predicting Skin Irritation	Utrecht, The Netherlands 9–11 November 1997	Van de Sandt <i>et al.</i> (1999) <i>ATLA</i> 27 , 723–743
39	Cell Transformation Assays as Predictors of Human Carcinogenicity	Angera, Italy 12–16 October 1998	Combes <i>et al.</i> (1999) <i>ATLA</i> 27 , 745–767
40	Biomarkers as Predictive Tools in Toxicity Testing	Burnham Market, UK 5–8 October 1998	Benford <i>et al.</i> (2000) <i>ATLA</i> 28 , 119–131
41	Three Rs Approaches in the Production and Quality Control of Avian Vaccines	Langen, Germany 11–13 June 1999	Bruckner <i>et al.</i> (2000) <i>ATLA</i> 28 , 241–258
42	The Second ECVAM Workshop on Phototoxicity Testing	Berlin, Germany 22–27 June 1999	Spielmann <i>et al.</i> (2000) <i>ATLA</i> 28 , 777–814
43	Novel Pyrogen Tests Based on the Human Fever Reaction	Konstanz, Germany 16–20 January 2000	Hartung <i>et al.</i> (2001) <i>ATLA</i> 29 , 99–123
44	The Establishment of Human Research Tissue Banking in the UK and Several Western European Countries	Birmingham, UK 8–10 September 2000	Anderson <i>et al.</i> (2001) <i>ATLA</i> 29 , 125–134
45	Novel Advanced <i>In Vitro</i> Methods for Long-term Toxicity Testing	Innsbruck, Austria 17–21 May 1999	Pfaller <i>et al.</i> (2001) <i>ATLA</i> 29 , 393–426
46	<i>In Vitro</i> Models of the Intestinal Barrier	Paris, France 4–5 March 1999	Le Ferrec <i>et al.</i> (2001) <i>ATLA</i> 29 , 649–668