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**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”

**Adopted by the CSTEE during the 41st plenary meeting
of 8 January 2004**

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BACKGROUND

Good quality and adequate information on adverse effects of exposure to chemical, physical and biological agents is essential in order to protect human health and the environment. The principal source of this information to date has involved the use of laboratory animal tests. For ethical, scientific and economic reasons, over thirty years there has been continuing intensive debate, and much research, as to how these animal tests can be reduced, replaced or refined, without compromising the high level of human health and environmental protection demanded by the European Community. A recent addition to this debate has been the published report of BUAV (British Union for the Abolition of Vivisection) - ECEAE (European Coalition to End Animal Experiments) on "The way forward - Action to end animal toxicity testing". The report proposes to end all animal toxicity testing in the context of the Commission proposal for the new chemicals legislation (REACH system)

Based on the mandatory information requirements for all chemicals (both new and existing) it is expected that a considerable number of animals may have to be used to satisfy these requirements for chemicals with an inadequate toxicological database. The BUAV-ECEAE report criticises the animal methods currently used for toxicity testing of chemicals, claiming that their credibility is based on established use rather than reliability or predictive value and that non-animal alternatives are already available to end animal testing for toxicity, without compromising in chemical safety. For fourteen endpoints the report suggests deficiencies of current tests and proposes alternative stepwise testing strategies, which they claim would avoid the use of vertebrate animals.

The CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment) was requested by DG Environment to assess the scientific quality of the report and to provide the scientific basis for its opinion. The CSTEE was also asked to comment on the adequacy of the non-animal methods proposed by the BUAV-ECEAE for classification and labelling and for the risk assessment of industrial chemicals. To perform this task, an inter-Committee Task Force has been established with members of the CSTEE, SCCNFP (Scientific Committee on Cosmetics and Non-Food Products intended for Consumers), SCMPMD (Scientific Committee on Medicinal Products and Medical Devices) and EFSA SC (Scientific Committee of the European Food Safety Authority). This opinion reflects the consensus view of this Task Force. The CSTEE adopted its opinion on the basis of the consensus view of the Task Force.

The Task Force considers the approach taken by the European Union emphasising the three Rs (reduction, refinement, and replacement) regarding animal experimentation in toxicity testing to be a relevant and appropriate action. The Task Force is of the opinion that alternative methods, when validated, should be used to replace animal experimentation in all instances when the ability to perform a scientific assessment of product safety is not compromised.

Toxicological testing aims to predict possible adverse effects in humans when exposed to chemicals. Currently it is extensively based on animal testing to identify hazards and the dose-

response relationship of chemicals. Ethical concerns have been raised by the use of laboratory animals. However, independent of ethical concerns, the primary objective of the risk assessment of chemical exposures is the protection of human health, wildlife and ecosystems.

As a background to assess the validity of the alternative methods and the proposed step-by-step testing strategies in the BUAU-ECEAE report, the Task Force made use of the ECVAM (European Centre for Validation of Alternative Methods) report titled "Alternative (non-animal) methods for chemical testing: current status and future prospects" which was published in 2002 (Worth and Balls, eds.) and of a number of publications listed below.

Terms of reference

Taking the ECVAM report as a background, DG Environment invites the CSTEE to examine the report provided by BUAU-ECEAE.

The CSTEE is invited to assess the overall scientific quality of the report. In considering this, the CSTEE is asked to comment on the reasoning and the conclusions presented in the report and to elaborate on the reasons for any divergence of opinion.

The CSTEE is particularly asked to comment on the adequacy of the non-animal alternative methods proposed by BUAU-ECEAE for each endpoint considered in the context of classification and labelling, and of risk assessment of industrial chemicals.

COMMENTS ON THE BUAU-ECEAE REPORT

GENERAL COMMENTS

The report describes the authors' view of the present status of toxicity testing for chemicals, states a number of criticisms of the present use of animal experimentation in toxicity testing and proposes a step-by-step testing strategy for chemicals and a time-frame for implementation of this strategy. The report claims that animal tests can be replaced by modern and humane alternatives.

The Task Force addresses a number of general points before commenting specifically on the fourteen endpoints of toxicity testing included in the report:

- The mechanism(s) of induction of adverse effects leading to endpoints such as cancer, genetic disease, reproductive disorders and allergies by exposure to chemicals involves complex biological interactions including multicellular, multiorgan, hormonal, neural, vascular and immunological systems. Modelling of such complex adverse effects cannot be accomplished, at present, by the use of non-animal tests. Specific toxic effects related to unexpected interaction/s of the different mechanisms will likely go undetected if reliance depends solely on non-animal tests.
- *In vivo* toxicological testing addresses both hazard identification and evaluation of dose-response relationship. The latter is an essential aspect of risk assessment. The BUAU-ECEAE report only addressed hazard identification. The omission of dose-response assessment is a serious deficiency in their report.
- Some of the *in vivo* test procedures mentioned in the BUAU-ECEAE report are already obsolete, infrequently used (e.g. classical LD-50 determination) and have been replaced by alternative methods requiring a reduced number of animals (fixed dose method, OECD 420, EC

B.1 bis; acute toxic class, OECD 423, EC B.1; up-and-down procedure OECD 425) or no animal experimentation (in the future for cosmetic ingredients) (Spielman and Liebsch, 2001)

- When criticising the presently used animal experimentation approach, the report does not take into account that the process of risk assessment does not use a single data set, rather it requires the combination of all the results from toxicity studies (including toxicokinetics and toxicodynamics) in order to reach valid conclusions. By this process, combined with expert knowledge, many of the critical issues in extrapolation of risks from animal experimentation can be addressed.
- Human health risk assessments rely heavily on the identification of NOAELs (no-observed-adverse-effect levels, or other surrogates for toxicity thresholds) for chemicals with dose thresholds, or risk-specific doses for chemicals without dose thresholds. These NOAELs and risk-specific doses are derived from long-term studies with laboratory animals. At present, there are no scientific grounds for the proposal that the *in vitro* testing strategy by itself can identify points relevant for risk assessments such as NOAELs or BMDs (Benchmark Doses). It is very unlikely that *in vitro* studies will be able to provide a sound basis for the identification of NOAELs or risk specific doses in the foreseeable future (Walton *et al.*, 1999; Holme and Dybing, 2002).
- The report gives general comments on the issue of risk assessment, but fails to consider specific aspects of risk assessment. The proposed computer models to predict distribution and metabolism require validation in animals and do not predict tissue concentrations of administered chemicals with sufficient accuracy nor do they predict target organs. Moreover, based on our current understanding of biological complexity, it is very unlikely that a few selected cell culture models could represent the multitude of potential target cells with respect to physiology, biochemistry, pathogenesis, capacity for biotransformation of chemicals and hence the sensitivity to toxic effects. Many chemicals show target organ specific responses in animal toxicity studies arising from a combination of a number of processes such as the accumulation of the chemical in the target organ, specific capacity of the target organ for biotransformation of the chemical, specific sensitivity of the target cells to the toxic effects of the chemical, and the complex and often poorly understood interactions of cells and their mediators in different tissues and organs that are influenced by the neuroendocrine and immune systems.

Since *in vitro* cell culture models cannot account for “unknown” mechanisms of action, which are detected in live animals (where all the relevant interactions occur), the predictive value of non-animal alternative tests is limited at present. The same is true for the proposed computer-based evaluations since these systems can only incorporate already existing knowledge into “expert systems”, but are unable to predict unexpected or unanticipated mechanisms of action.

- The report criticises the “scientific failing” of the established methods, but does not discuss the possible additional problems related to the reliability and predictive value of the “non-animal”-strategies put forward. The report does not acknowledge the fact that the problem of extrapolation from a controlled experimental situation to varying human exposure and response(s) are even greater for non-animal assays. The non-animal alternatives that are available are much simpler test systems than a complex mammalian organism and the selected use of non-animal experimentation will as a consequence cause a significant increase in uncertainty. Even testing strategy using an array of *in vitro* assays requires additional extrapolation to assure that all possible endpoints have been addressed. Thus *in vitro* assays will only be able to address specific endpoints for which the relevance has already been demonstrated in *in vivo* toxicity.

- The report claims that the presently used animal testing strategy for chemicals is highly uncertain, that the test systems used are of dubious scientific value and that their credibility is based on established use rather than reliability or predictive value. In addition, the report claims that none of the animal test systems used at present in toxicity testing has undergone a formal validation process. The predictive value of animal testing is based on very similar biochemical and physiological aspects between test animals and humans, and on the large (and very good) database/s available. The report overlooks the fact that:
 - frequent use of such tests in numerous laboratories is giving consistent results and most animal testing methods have been validated by OECD (Organisation for Economic Co-operation and Development).
 - the safety of human medicinal products on the EU market is considered to be adequate and is primarily based on toxicity testing in animals before the first human trials are initiated. In addition, animal tests are used for assuring biocompatibility of medical devices.
 - all human carcinogens established by epidemiology are animal carcinogens; when quantitative comparisons are possible, even a prediction of carcinogenic potency (dose strength) based on animal data is possible (Allen *et al.*, 1988).
 - the scientific literature abounds with examples demonstrating that animal models are good predictors for chemically-induced disorders in humans (Sipes *et al.*, 1997; Klaassen, 2001; Hayes, 2001). There may be differences in responses between animals and humans, but they are most often of a quantitative, rather than a qualitative nature (Dybing *et al.*, 2002). It is true that some chemicals induce toxic effects in animals that are not seen in humans (Holden and Tugwood, 1999; Roberts, 1999; Roberts *et al.*, 2002; Swenberg and Lehman-McKeeman, 1999; Cook *et al.*, 1999) and that some chemically-induced diseases in humans have not been modelled in animals (Tatu *et al.*, 1998); however, these are the exceptions rather than the rule.
- The Task Force would like to stress that the considerations developed in this opinion apply equally for chemicals used for food and natural compounds.
- A reliance on non-animal testing of chemicals will increase the uncertainty of extrapolating toxicity data to humans, thus reducing the objective of ensuring chemical safety. A stronger emphasis on non-animal testing may also lead to demands for safety testing in humans.
- The BUAV report surprisingly only considers mammalian toxicity. However, the REACH initiative is broader in its scope and also addresses a reduction in non-mammalian animal testing (e.g. ecotoxicological assays involving vertebrates and invertebrates). The task Force notes that the ECVAM report is also limited to mammalian toxicity.
- Mammalian toxicity tests are also needed in the environmental risk assessment, and additional animal testing is conducted for the ecotoxicological evaluation. The Task Force has considered it appropriate to comment also on the use of animal testing for assessing effects on wildlife and ecosystems. This approach is in line with the recommendation for an integrated Risk Assessment Strategy covering human health and environment aspects.
 - Ecotoxicological testing usually includes tests on vertebrates such as fish and birds, several terrestrial and aquatic invertebrates, algae, terrestrial and aquatic plants and microbial populations. Testing in mammals is not frequent in ecotoxicology; however most of the toxicity tests conducted for the human health assessment are also used for the ecotoxicological assessment. The test results must be reassessed to identify ecologically relevant endpoints, such as those related to survival, growth and reproduction. Thus, the

NOAEL employed for human health and the NOEC (no-observed-effect concentration)/NOEL (no-observed-effect level) employed in ecotoxicology can be different even if obtained from the same test.

- Toxicity tests on non-mammalian vertebrates cover mostly birds and fish, although testing on amphibians is presently receiving additional attention, mostly within the testing strategies for endocrine disrupters. The most important tests on birds are those measuring reproductive effects, and there are currently no *in vitro* alternatives.
- Fish are considered as representing an essential species for assessing effects on aquatic ecosystems. *In vitro* tests on fish cell lines have been developed, tested and proposed as alternatives to acute toxicity tests for over 20 years (Anhe *et al.*, 1985; Babich *et al.*, 1986). The possibilities and limitations of these tests have been discussed elsewhere (i.e., Castaño *et al.*, 1996; Segner 1998; Dayeh *et al.*, 2000; Fenk, 2001). Some sublethal effects can also be assessed *in vitro*, including enzymatic induction and endpoints for identifying endocrine effects. However, these systems cannot quantify the potency and ecological relevance of the observed effects. Whilst they can be helpful for the screening and initial steps of hazard identification, they cannot replace the *in vivo* tests in the foreseeable future.
- Some *in vitro* tests on invertebrates have also been developed and used for ecotoxicological testing (Birmelin *et al.*, 1996; Braeckman *et al.*, 1997), but at present their use is sporadic since they cannot be considered as valid alternatives to *in vivo* methods.
- It must be appreciated that the purpose of ecological testing is to protect ecological systems; i.e. collective groupings of organisms (such as populations). Effects at suborganismic levels are unlikely to give adequate information on ecological responses, and we know too little about ecosystems to model them effectively from an ecotoxicological point of view. Therefore, the Task Force is of the opinion that currently *in vivo* testing is an essential need for ecotoxicology and ecological risk assessment. The possibilities offered by *in vitro* alternatives should be carefully assessed and scientifically developed and validated before possibly replacing the current *in vivo* systems, including the tests on mammals employed simultaneously in the human and environmental assessments.

SPECIFIC POINTS

Eye and skin irritation and corrosion

In the ECVAM report, these topics are summarised in a chapter on “Local toxicity: acute dermal and ocular effects”; the BUAV document separates this topic into eye and skin irritancy.

The BUAV proposes a step-by-step testing using QSAR, chemical reactivity and *in vitro* tests to replace the animal toxicity tests used for these endpoints. In the ECVAM report, a tiered testing is also proposed focusing mainly on a number of *in vitro* methods.

It is in the area of local toxicity that most progress has been made, over the past two decades, in the development of non-animal procedures. It should be noted that for local toxicity, toxicokinetics including metabolism generally play only a minor role. A tiered testing strategy is recommended by the OECD and adopted by the EU. The Task Force also supports a stepwise approach for testing of local effects, focusing on the use of *in vitro* methods. For confirmation of absence of local irritating effects, limited animal testing, however, may still be necessary to obtain the required certainty for the classification and labelling process and to demonstrate the absence of irritating effects.

Eye irritation

Despite major efforts by ECVAM, industry and academia, no validated replacement test for the Draize rabbit eye irritation test (OECD 405, EC B.5) is available at present. Six major validation studies were completed between 1991 and 1997 (Balls *et al.*, 1995; Brantom *et al.*, 1997; Spielmann *et al.*, 1993, 1996; Gettings *et al.*, 1991, 1992, 1994, 1996; Bradlaw *et al.*, 1997; Ohno *et al.*, 1994). The non-animal tests currently available comprise physicochemical tests, cell and tissue culture systems and organotypic models (Christian and Diener, 1996; Chamberlain *et al.*, 1997; Spielmann *et al.*, 1997). They are screening tests to detect an irritating potential rather than definitive tests. The Task Force agrees that they are useful to distinguish between strong irritants and non-irritants, but they frequently fail to make a reliable distinction between non-irritative and moderately irritating substances. Often recovery of the injury is not being measured in the *in vitro* tests.

In a number of national regulatory agencies in Europe some of the existing *in vitro* tests (e.g. BCOP, IRE, CEET, HET-CAM) are accepted for specific and limited purposes (Worth and Balls, 2002). Confidence in the results is then mainly dependent on the availability of appropriate benchmarks, information on related substances, a proper understanding of the limitations, and the expertise of the user.

Within industry, a number of these tests are currently used e.g. to screen for strong irritants; for the cosmetic industry their use is in particular related to finished product testing.

Skin irritation

Non-animal tests currently available include simple to complex organotypic cultures and reconstituted human skin models (Botham *et al.*, 1998, van de Sandt *et al.*, 1999). From a prevalidation study carried out during 1999-2001 and additional follow-up activities (Fentem *et al.*, 2001) only three tests (Episkin, Epiderm and SIFT) will be the subject of a formal validation in 2004 (Fentem and Botham, 2002). The Task force awaits the outcome of this validation exercise with great interest.

In the cosmetic industry, non-animal skin irritation tests are currently applied to test skin compatibility of finished products, but not to assess the safety of cosmetic ingredients. Such tests are considered to be an intermediate step before commencing compatibility testing on a small group of human volunteers.

The absence of skin irritation for cosmetic ingredients can be confirmed on human volunteers only in a limited number of cases (SCCNFP Guidelines for human testing in cosmetic science). As part of its strategy the BUAV report proposes that skin irritation testing of chemicals be performed on human volunteers in those Member States where it is allowed. This raises important ethical questions.

Skin allergy and sensitisation

Both the BUAV and ECVAM propose a tiered strategy that is mainly based on the use of *in vitro* methods to replace the Buehler- and the Guinea-pig maximisation test. The BUAV does not consider respiratory sensitisation, which is a major problem when handling chemicals in the occupational setting. The tiered strategies proposed by ECVAM and BUAV are not very different in respect of the use of non-animal tests. ECVAM, however, supports the use of procedures based on animal experimentation to confirm the absence of a potential for sensitisation *in vivo* when all *in vitro* testing gives negative results. The animal test designated as the local lymph node assay (LLNA) in the mouse has been adopted by ESAC (ECVAM Scientific Advisory Committee) as a valuable refinement test when compared to the current practice of using the guinea pig (Basketter, 2002; EN ISO 10993-10). An important advantage of the LLNA (mentioned by ECVAM but not by BUAV) is that besides its use for hazard identification, this test can also be used for the determination of relative potencies (Kimber *et al.*, 2002; Basketter, 2002).

The Task Force does not agree with the criticisms of BUAV on animal tests used for skin allergy and sensitisation. Despite the use of often large doses of a potential allergen, the use of inbred animals, and the differences between the microstructure of the test animal's skin and the human, there is a good correlation between *in vivo* animal test results and human data (Kimber *et al.*, 2002).

The Task Force agrees with ECVAM that no *in vitro* system available at present, or expected to be available in the near future, is sufficiently reliable to predict all aspects of sensitisation. Thus, the Task Force also supports the approach taken by ECVAM proposing limited animal testing for chemicals negative in the non-animal assays.

Acute toxicity and repeat-dose toxicity

BUAV claims that a tiered *in vitro* only strategy may be able to predict both the acute and the repeated-dose toxicity of a chemical, not only in qualitative, but also in quantitative terms. The strategy proposed involves computer-assisted recognition of "toxophores" (structural elements in a chemical which are associated with toxic responses) and toxicity testing in cell culture. In this respect the recommendations made by the BUAV for proceeding in this direction are ill-defined and, especially regarding the quantitative aspects, are not scientifically substantiated.

ECVAM also proposes a tiered approach with decision points, some based on computerised prediction of toxicity using QSAR, prediction of biotransformation pathways and cell specific assays for the assessment of acute and repeated dose toxicity. However, ECVAM concludes that none of the proposed *in vitro* models has been validated for reliability and relevance. ECVAM therefore suggests that *in vitro* tests may be helpful to replace the dose-range finding studies for a final *in vivo* toxicity testing to reduce the number of animals used.

At the present time, the Task Force does not consider that it is scientifically justified to replace the presently recommended fixed-dose or acute toxic class methods. These tests use few animals and dose-range finding can be supported by computerised prediction and expert involvement. In addition, well performed acute toxicity assays give information on the onset and duration of toxic responses and may also yield information on both potential target organs and treatment of human intoxications. The use of methods involving animals also permits conclusions for classification and labelling regarding toxic potency. None of the *in vitro* assays available at present can accurately predict potency in mammals.

A further problem with the application of *in vitro* methods is that a combination of different methods always has to be used in an attempt to cover the many possible mechanisms of acute lethality (e.g. interference with neurotransmitter function or energy metabolism, necrosis due to interaction

of reactive intermediates). Moreover, the results from the *in vitro* test methods require complex and unreliable extrapolations, which hamper overall predictivity and reliability.

The BUAV report suggests replacing repeat-dose testing using the same *in vitro*-only strategy and claims that potency and points of departure for risk assessment such as NOAELs and BMDs may also be derived from the *in vitro*-only approach. However, as discussed above, the toxic response is typically multifaceted and depends on a multitude of factors such as the physicochemical properties of a chemical, the routes and duration of exposure, the toxicokinetics and toxicodynamics. On current understanding, effects can only be established in intact animals to assess repeat-dose toxicity and establish NOAELs. The available non-animal methods cannot account for toxicokinetics *in vivo* (Blaauboer, 2002). The proposed BUAV strategy to overcome these problems is not scientifically substantiated in the report and no attempts are made to propose an approach to obtain quantitative data. The Task Force also notes that other important aspects, such as recovery studies and gender variability, were not considered in the report.

ECVAM does not propose a strategy for repeat-dose testing, but concludes that due to the complexities of endpoints of toxicity, the use of *in vitro* approaches is very difficult due to the lack of availability of suitable *in vitro* systems.

The Task Force concludes that at present the complex interactions occurring in the toxic response(s) *in vivo* are very difficult, if not impossible, to mimic and predict using individual or combinations of *in vitro* systems. This is often due to inadequately defined mechanisms of action, which are used to develop *in vitro* systems. As a consequence, any approach using non-animal testing only will not have the reliability needed as the basis for informed decisions. The absence of methodologies to obtain points of departure for risk assessment (such as NOAELs, BMDs) is a major obstacle for the use of the non-animal strategy.

It should be recognised that mammalian tests are also employed in environmental risk assessment for assessing secondary poisoning. The ecotoxicological assessment requires the specific quantification of ecologically relevant endpoints: survival, growth and reproduction. The current state of the science does not allow the quantitative estimation of LD50s and NOAELs from *in vitro* tests, and this is particularly relevant for multifactorial responses such as those required in ecotoxicology.

Mutagenicity and carcinogenicity

In the BUAV report, a large number of criticisms are put forward on the animal testing procedures for these endpoints. Most of the criticisms are based on the problem of extrapolation from a controlled experimental situation to different and variable human exposures and responses. However, extrapolation to human situations based on the use of the proposed non-animal assays will be even more difficult. This issue is not addressed in the report.

Regarding mutagenicity, the BUAV proposes to use computer-based systems in combination with established *in vitro* test systems that are already widely used for assessment of genotoxic hazard. The ECVAM report also focuses on the use of validated *in vitro* testing for hazard assessment with regard to genotoxicity, but also includes an *in vivo* test when ambiguous results are obtained. The ECVAM report proposed to use toxicokinetic data and a validated germ-cell mutagenicity assay for classification of germ-cell mutagens.

The Task force acknowledges that in the area of genotoxicity testing, substantial progress has been made over the past forty years resulting in the availability of a number of non-animal methods, and these systems are routinely used for hazard assessment and in the classification and labelling process.

The Task Force supports the approach proposed by ECVAM. It is current practice to use the well-established *in vitro* systems to the largest extent possible. Based on a recent consensus completed by a group of experts from universities, institutions and industries, regarding hazard assessment of genotoxic/mutagenic chemicals, *in vitro* genotoxicity/mutagenicity data from an elementary set provide information on i) gene mutations, ii) structural chromosome aberrations, and iii) numerical chromosome anomalies. At present, however, the three elementary genetic end-points cannot be adequately covered with a single test system. This elementary set of genotoxicity *in vitro* data is considered appropriate whatever the area of use of the tested chemicals or the human exposure/dose scenario (Muller *et al.*, 2003).

The relationship between the results obtained from genotoxicity/mutagenicity testing and genotoxic risk for exposed human populations is still under discussion, for example the need of confirmatory *in vivo* tests, as compared to the quality and the relevance of the *in vitro* test results; the existence of a threshold for aneugens; the explanation of a non-linear dose-response for clastogens and non-complementary results from *in vitro* genotoxicity studies based on the same end-point.

The Task Force considers that confirmatory, *in vivo* tests are still necessary in order to assess any genotoxic risk for exposed human populations. The need for and the choice of *in vivo* mutagenicity tests should be justified by the results obtained with the *in vitro* tests. Before any *in vivo* testing is performed, a review of the *in vitro* test results should be required. A particular *in vivo* mutagenicity test should be conducted only when it can be reasonably expected from the properties of the test substance and the test protocol that the specific target tissue will be adequately exposed to the test substance and/or its metabolites. If necessary, an assessment of the toxicokinetics should be conducted before progressing to *in vivo* testing. In the case of a positive result *in vitro*, the *in vivo* mutagenicity test should use the same end point and target tissue, if possible.

Regarding carcinogenicity, BUAV proposes to use computerised prediction, cell transformation assays and mechanistic *in vitro* studies. The BUAV does not mention that a well performed carcinogenicity bioassay in animals will in many situations also be a chronic toxicity assay since the two endpoints can be combined. ECVAM also proposes a tiered approach to carcinogenicity testing, which in fact is already used in industry for the assessment of a potential carcinogenicity of a chemical during the selection of products for development. The proposed approach involves cell transformation assays (OECD draft guidelines for testing of chemicals), genotoxicity testing, QSAR, but also a classical *in vivo* test when the data generated initially are negative or ambiguous.

The scheme proposed by ECVAM is already used as an approach to predict carcinogenicity and the conclusions for carcinogenicity based on genotoxicity are valid. Since most potent mutagens are also carcinogens in animal experiments it has to be questioned why a carcinogenicity bioassay should be required when a chemical is consistently genotoxic in the *in vitro* test battery. In such cases, the results of testing will be useful to determine potency and target organs and can also have important consequences for classification and labelling. In addition, data from both genotoxicity and carcinogenicity testing are essential for a quantitative risk assessment for exposure to carcinogens and to set priorities for the regulation of chemicals. Regarding identification of NOAELs or risk-specific doses *in vitro* tests cannot provide the crucial data.

Teratogenicity

Although BUAV has only considered teratogenicity (i.e. malformations), assessment of developmental toxicity tests should also include the endpoints of embryotoxicity and foetotoxicity (e.g. reduced foetal growth).

BUAV proposes the use of structure-activity analysis by computer programmes and three *in vitro* test systems (embryonic stem cell tests, micromass test and postimplantation assay). BUAV

claims that by this strategy teratogens can be clearly identified and they propose a time scale of only two years for the implementation of the “non-animal systems”.

In contrast, ECVAM stresses that a tiered strategy is needed to address a potential for teratogenicity and that two of the proposed “*in vitro* assays” (micromass test and postimplantation rat whole embryo test) also require a large number of pregnant animals. The third assay is the embryonic stem cell test (EST), which has shown to be a good predictor for strongly embryotoxic chemicals (Genschow *et al.*, 2002). A recently organized workshop (ECVAM embryotoxicity workshop, ECVAM 28-30 January 2003) aimed at the identification of realistic applications of the validated *in vitro* test, and discussed the limitations and the future steps necessary to gain regulatory acceptance of these. It concluded that at the current stage of validation, these tests cannot be utilised for regulatory purposes. Among the important limitations identified were: i) the limited number of chemicals included in the study, i.e. 20; ii) important chemical classes were not included, especially industrially relevant compounds; iii) the potent developmental toxicants represented a limited number of mechanisms of toxicity, mostly affecting cell proliferation; and iv) the absence of metabolic systems in the assays. The workshop report noted that several important classes of industrial chemicals require metabolic conversion for developmental toxicity to occur, for example phthalates and glycol ethers.

The BUAV report does not mention that the *in vivo* database established over decades gives strong evidence regarding the predictive value of animal tests. To date, with the exception of a few chemicals, e.g. the coumarin anticoagulant drugs, all chemicals identified as human teratogens have been shown to be teratogenic in one or more laboratory animal species.

ECVAM explicitly states that animal tests are still required for assessment of teratogenicity due to the complexity of the response, a limited understanding of the mechanisms involved and often unknown structural requirements for teratogens.

The Task Force considers the BUAV approach and the proposed time-frame for implementation of their strategy as unrealistic. Moreover quantitative animal data are necessary in order to perform risk assessments and to derive guidance values. At present, discontinuation of animal testing for developmental toxicity will therefore result in compromised chemical safety.

Reproductive toxicity

BUAV proposes to use a combination of the physicochemical properties of the compounds, a computerised screening for structural alerts and *in vitro* tests for the prediction of reproductive toxicity. No specific recommendations are made. In contrast, ECVAM considers that the development of *in vitro* tests to model the reproductive cycle is still at a very initial stage. Since it will take time to develop and validate a battery of alternative tests that can cover the various aspects of the reproductive cycle, animal tests will continue to be needed. Thus the present ECVAM recommendation is to review the existing tests in order to refine the methods to reduce the number of animals required.

In addition, within the DG RTD 6th Framework Programme a new strategy has been put forward by ECVAM in collaboration with OECD, to develop and evaluate a set of alternative tests covering the different endpoints in the field of reproductive toxicity.

The Task Force agrees with the conclusions of ECVAM and stresses that, due to the complex nature of mammalian reproduction, non-animal assays are likely to be less reliable and of lower predictive value than traditional animal tests for hazard identification. Again, such non-animal tests will be of little value for hazard characterisation and risk characterisation.

As mentioned for acute and repeat-dose toxicity, it should be also pointed out that mammalian reproduction tests are essential for assessing the risk of secondary poisoning, including biomagnification processes. In contrast to the human health assessment, ecotoxicological assessments do not focus on individuals, but on populations. Therefore, these assessments require the estimation of effects on reproduction at the population level, which cannot be identified from *in vitro* tests. This information is essential for assessing the risk of chemicals on wild mammalian populations, including endangered species.

Toxicokinetics

To predict toxicokinetics, the BUAV report proposes a combination of *in vitro* studies and computer simulations. A detailed approach to the problem is not presented and the priority action is not focused on the problem since toxicokinetics do not measure direct health effects, but are part of the toxicology testing strategy. The ECVAM report gives an overview of the many *in vitro* systems already used for specific purposes in the area of toxicokinetics (referred to as biokinetics in the BUAV report). A large number of these *in vitro* systems are widely used in research and in the development of new chemicals by industry. There are a number of computer models available that can predict, to varying extents, biotransformation pathways and that can simulate the toxicokinetics of a chemical in animals. They can also assist with the extrapolation of animal data to humans. However, the conclusions that can be derived from these systems are limited and, at present, a reliable, exclusively non-animal strategy does not exist. If at all possible, it would need to be based on a complex combination of *in vitro* studies. The Task Force advises that much more work is needed before the practicality of the proposed BUAV strategy can be assessed. It notes that a conclusive toxicokinetic study in animals can be performed with a low number of animals using various doses of a chemical. This provides all the relevant information needed in a short time.

Endocrine disrupters

The Task Force emphasises that endocrine disruption is not a toxicological endpoint *per se*, but is one of many mechanisms of action that may lead to various types of health impairment and disease. The BUAV report again uses a stepwise strategy for endocrine disrupter testing. The approach mainly focuses on oestrogens and androgens, and includes *in vitro* receptor binding assay and oestrogen responsive genes. The BUAV report does not recognise that endocrine disruption may be a complex process not only involving oestrogens and androgens, but a variety of other hormones and a variety of interactive mechanisms. The endpoints of relevance in this respect, such as thyroid or adrenal dysfunction, or diseases related to disturbances of hormonal balance *in vivo* due to an interaction of a chemical with the biosynthesis, disposition or catabolism of hormones, would go undetected by the proposed approach.

In addition, BUAV claims that data relevant to endocrine disruption will be available when chemicals have undergone testing for a variety of other toxicity endpoints using the *in vitro* approach. While this may be correct when using animal experimentation, only limited information on endocrine related endpoints can be extracted from the many specific *in vitro* assays proposed in the non-animal testing strategy. In the *in vitro* assays, proposed or available endpoints addressed are usually not hormone-dependent; thus information relevant to endocrine disruption is unlikely to be achieved.

The ECVAM report recognises that mechanisms of actions of the so-called endocrine disrupters are complex and diverse and that any testing scheme using *in vitro* assays only will have to include a large number of specific endpoints. Animal experimentation is still considered as mandatory in the test system to obtain reliable results, and non-animal approaches are only applicable for prioritisation for further testing. Under the umbrella of the OECD, *in vivo* tests, such as the enhanced 407 repeated-dose oral toxicity test in rodents, are currently being validated. When

validated, the enhanced OECD 407 will be presumably a refinement compared to the existing assay. Preliminary results indicate that this test is also promising for hazard identification and dose-response assessment of chemicals showing endocrine disrupter effects. A repeat-dose oral toxicity test is a base-set requirement in the EU for new chemicals.

On scientific grounds, the Task Force is unable to support the BUAV report's proposal to use solely *in vitro* assays for predicting *in vivo* endocrine disrupter effects, since they only cover sex hormones, would not address all possible mechanisms and often generate false-negative results. However, *in vitro* assays may be useful in setting priorities for further testing and for supplying information for the understanding of the mode of action.

Use of non-animal testing procedures in the context of Classification and Labelling (according to Annex VI to Directive 67/548/EEC, as last amended by Directive 2001/59/EC)

When performing classification on toxicological endpoints, most of the procedures are based on both qualitative and quantitative data obtained in guideline animal tests. These tests are reliable and predictive, and points of departure can be derived for risk assessment.

In our current state of knowledge *in vitro* tests cannot generate the data required for Classification and Labelling. Many endpoints used for Classification and Labelling are not addressed with sufficient reliability by *in vitro* testing. Some of the data required for Classification and Labelling involve the need to obtain quantitative information that cannot be generated by the use of *in vitro* tests.

Developing new Classification and Labelling procedures based on *in vitro* tests is in principle possible, as the Classification and Labelling systems are basically strategies for ranking chemicals. However, the level of hazard identified by the different *in vitro* tests compared to that identified by *in vivo* tests in terms of human health and environmental protection, are unknown.

The Task Force notes that the current Classification and Labelling procedures are based on already established levels of hazard for human health and the environment; and are used as starting points not only for risk assessment but also for down-stream legislation, such as worker protection legislation or the Seveso II Directive.

Use of non-animal testing procedures in the context of risk characterisation

Risk characterisation is an essential process for ensuring a high level of safety from chemical exposures to man and the environment. It involves the integration of exposure assessment and hazard characterisation steps in an overall risk assessment. Central for the hazard characterisation step is the identification of the critical effect and its dose dependency. For non-genotoxic agents threshold surrogates (NOAEL, BMD) are identified from long-term animal experiments (including structural and functional chronic toxicity, and reproductive toxicity). For genotoxic carcinogens dose indicators are identified as the starting point from *in vivo* carcinogenicity studies.

Given the very complex physiological and pathological interactions involved in chronic toxicity, reproductive toxicity, carcinogenicity and sensitisation, risk characterisation is at present not possible without the use of animal test systems.

SUMMARY

- This opinion was adopted by the CSTEE on the basis of the consensus view of the Task Force which included members of the CSTEE, SCCNFP (Scientific Committee on Cosmetics and

Non-Food Products intended for Consumers), SCMPMD (Scientific Committee on Medicinal Products and Medical Devices) and EFSA SC (Scientific Committee of the European Food Safety Authority)

- Alternative methods, when validated, should be used to replace animal experimentation in all instances where the ability to assess chemical safety reliably is not compromised. Progress on development and validation of non-animal tests is noted and strongly encouraged.
- It is further noted that very substantial progress has already been made in non-animal testing in respect of genotoxicity testing and in the assessment of local toxicity. Several other promising developments have been made, which will require full validation before they are considered as replacements for existing *in vivo* tests. One criterion must be that the present high level of public health and environmental protection, which is founded on results from current testing methods, is not compromised
- In respect of the overall scientific quality of the report “The way forward - Action to end animal testing” of The British Union for the Abolition of Vivisection (BUAV) and the European Coalition to End Animal Experiments (ECEAE), the conclusions and recommendations tend to be based on unsubstantiated assertions rather than scientific evidence. Thus, in many instances, the reasoning and conclusions in the report are not justified by the available scientific data.
- It is emphasised that to perform a sound risk assessment, an understanding of dose-response relationship is essential. It is unfortunate that the BUAV-ECEAE report almost exclusively focuses only on methods for hazard identification. Moreover, many of the non-animal alternative methods proposed in the report for the various toxicity endpoints are also inadequate to perform hazard identification.
- The report fails to recognise the complexity of biological systems and the importance of the interaction between physiological pathways which may only occur *in vivo*. A number of important issues are not addressed in the BUAV report, such as the use of mammalian toxicity data for the protection of wildlife and ecosystems.
- It is concluded that, for the foreseeable future, the use of live animals in toxicity testing is essential in order to perform reliable risk assessments.
- If adopted, the proposal by BUAV-ECEAE would lead to a significant reduction in the current levels of human health and environmental protection.

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Guidelines on the use of human volunteers in compatibility testing of finished cosmetic products [SCCNFP/0068/98].

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GLOSSARY

3 R	Reduction, Refinement, and Replacement
adverse effect	Change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. (IPCS, 1978)
BCOP	Bovine Corneal Opacity and Permeability test
BMD	Benchmark Dose Method
CEET	Chicken Enucleated Eye Test
EST	Embryonic Stem Cell Test
GPMT	Guinea-pig Maximisation Test
HET-CAM	Hen's Egg Test-Chorio Allantoic Membrane
IRE	Isolated Rabbit Eye Test
LD-50	Median Lethal Dose 50% : a statistically derived single dose of a substance that can be expected to cause death in 50% of the dosed animals (expressed in mg/kg body weight)
LLNA	Local Lymph Node Assay
NO(A)EL	No Observed (Adverse) Effect Level : the highest dose or exposure level within a specific test system, where no (adverse) treatment-related findings are observed
NOEC	No-observed-effect concentration
QSAR	Quantitative Structure-Activity Relationships
REACH	Registration, Evaluation, Authorisation and Restrictions of chemicals
SIFT	Skin Integrity Function Test

Organisations/Committees

BUAV	British Union for the Abolition of Vivisection.
CSTEE	Scientific Committee on Toxicity, Ecotoxicity and the Environment
DG RTD	(European Commission) Directorate-General Research
ECB	European Chemicals Bureau
ECEAE	European Coalition to End Animal Experiments
ECVAM	European Centre for Validation of Alternative Methods
EFSA	European Food Safety Authority

EPA	The United States Environmental Protection Agency
ESAC	ECVAM Scientific Advisory Committee
IPCS	International Programme on Chemical Safety
OECD	Organisation for Economic Co-operation and Development
SCCNFP	Scientific Committee on Cosmetics and Non-Food Products intended for Consumers
SCMPMD	Scientific Committee on Medicinal Products and Medical Devices