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**SCIENTIFIC COMMITTEE ON HEALTH AND ENVIRONMENTAL RISKS**  
**SCHER**

**Opinion on**

**“Endocrine Disrupting Chemicals: a Non-animal  
Testing Approach”  
(BUAV report - 2004)**

Adopted by the SCHER  
during the 8<sup>th</sup> plenary of 25 November 2005

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## 1. BACKGROUND

The Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) published in March 1999 a report on human and wildlife health effects of endocrine disrupting chemicals with emphasis on wildlife and on ecotoxicology test methods.

Due to the international concern that chemicals may interfere with the endocrine system of humans and wildlife, the Commission adopted a Community Strategy on Endocrine Disrupters in December 1999<sup>1</sup>. The Strategy particularly focuses on establishing a priority list of substances for further evaluation, monitoring, research as well as on the review and adaptation of Community legislation<sup>2</sup>.

With regard to legislative measures on endocrine disrupting chemicals, the question, how to assess their human and wildlife effects appropriately is of special interest. In any case it is a clear aim to avoid unnecessary animal testing.

The British Union for the Abolition of Vivisection (BUAV) submitted a report on a non-animal testing approach for endocrine disrupting chemicals to the Commission. The findings, conclusions and recommendations given in the report are particularly linked to directive 67/548/EEC for classification, packaging and labelling of dangerous substances and to the proposal for a new chemicals policy (REACH).

## 2. TERMS OF REFERENCE

The SCHER is invited to comment on the findings, conclusions and recommendations of the BUAV report in particular on the proposals and recommendations given in the report for a step wise non-animal testing approach for assessing endocrine disrupting effects in humans and wildlife.

## 3. OPINION

### 3.1. Introduction

The BUAV-document claims that the hazard characterization requirements to assess endocrine system mediated toxicities of chemicals in animals within the proposed new chemicals policy of the European Commission (REACH) may be achieved without animal testing. The BUAV-document proposes an *in-vitro* only approach applying computational methods (*in silico* toxicology such as QSAR and toxicokinetic modelling), hormone receptor binding assay using transcriptional activation, and “cell and molecular tests”. According to the statement of the BUAV-document, this approach is postulated to correctly identify “endocrine disrupting chemicals”.

The position of the SCHER relating to the use of animal testing in the context of the assessment of hazards and health risk assessment of chemicals, the 3R-concept of replacement, refinement and reduction of animal use for experimental purposes, and the

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<sup>1</sup> COM(1999)706

<sup>2</sup> [http://europa.eu.int/comm/environment/endocrine/index\\_en.htm](http://europa.eu.int/comm/environment/endocrine/index_en.htm)

continuation of the high level of human health and environmental protection is identical to the position of the former CSTE. This position is outlined in detail in comments by the CSTE (2004) on a previous report of the BUAV and will not be repeated here.

### 3.2. General comments

First, the SCHER wants to reemphasize that “endocrine disruption” is not a toxicological endpoint per se, but is one class of the many mechanisms of action that may lead to various types of effects in different species, which may result in adverse consequences on humans and ecosystems.

The present BUAV-document, in a very similar way as the previous BUAV-document titled “The way forward – Action to end animal testing” describes the present status of animal tests to assess toxicities on the endocrine system, states a number of criticisms of the use of animals in testing for toxicities (in this case focusing on toxicity to the endocrine system) and proposes a testing strategy based solely on *in-vitro* systems and computational toxicology. It is claimed that the application of this strategy will more rapidly, more reliably and more cheaply identify chemicals with possible endocrine effects.

The SCHER reiterates a number of points already elaborated in detail in the previous CSTE opinion (2004) before commenting specifically on the proposed strategy on endocrine effects assessment:

- The complex mechanisms of induction of adverse effects including a possible interference of a chemical with hormone receptors, chemically induced changes in the activities of the enzymes involved in hormone biosynthesis and hormone disposition, and interactions between different hormones are only incompletely understood and thus can, at present, not be modelled (Vos *et al.* 2000). For many non-mammalian species, particularly invertebrates, the current understanding of the endocrine system is even more limited as compared to mammals, making predictions of hazard and risk on the basis of *in-vitro* tests even more uncertain. Possible specific toxicities will likely go undetected if reliance depends solely on non-animal tests.
- Both hazard assessment and dose-response assessment are important aspects to be addressed in toxicity testing. Dose-response is essential in the process of effects assessment of humans and ecosystems (Holme and Dybing 2002). The omission of dose-response assessment in the approach selected by the BUAV-report represents a serious deficiency.
- A number of other points regarding the use of animal testing, the scientific value of the animal testing approach and possible additional problems related to the reliability and predictive value of the non-animal tests and the need for ecotoxicological testing are outlined in the CSTE opinion (2004) and also are major points of criticisms regarding the present BUAV-report. The opinion of the SCHER on these issues is identical to that of the former CSTE.

### 3.3. Specific comments

The BUAV proposes a stepwise *in-vitro* approach to identify chemicals with possible endocrine dependent toxicities. In a first step, BUAV-report proposes that QSAR shall

identify priority chemicals, which should then be subjected to receptor binding assays and a characterization of their effects on cultured cells. BUAV-report further proposes that these assays should be supplemented by performing further *in-vitro* tests (such as intestinal barrier models) and kinetic modelling to predict *in-vivo* disposition and biotransformation of the chemical and expected toxicities without animal testing.

Important details of the non-animal testing approach proposed by the BUAV-report are only vaguely described. The application of the BUAV testing scheme will only give information on the potential of a chemical to interact with the endocrine system, but only very limited information on types and characteristics of possible adverse effects in animals and humans. The potential for interaction of a chemical with some endocrine function in an *in vitro* test system alone should not be used as a basis for predicting adverse effects in intact animals, which may or may not be the consequence of this interaction (Gelbke *et al.* 2004).

Moreover, the report does not address the issue of how to use the information generated by the non-animal approach in the process of health risk assessment, which relies heavily on the determination of points of departure (NOAELs, benchmark doses) for the extrapolation process from results of toxicity testing in animals (Holme and Dybing 2002; Meyer 2003).

The BUAV-document also fails to mention the important role of *in-vitro* tests in currently proposed testing strategies for “endocrine disruptors”. The US EPA as well as the conceptual framework of the OECD integrate non-animal test systems (such as QSAR, physicochemical properties, information on human exposure, binding affinity to different hormone receptors and transcriptional activation) to prioritize chemicals for further testing with regard to toxicities to the endocrine system (Gray *et al.* 2002). Both programs, however, also rely on toxicity testing in animals with the ultimate aim to use these data in risk assessment. In line with the position of SCHER, the US EPA and the OECD considered the use of animal experimentation as essential due to the limitations of the *in-vitro* assays:

- *In-vitro* assays may not mimic *in vivo* modes of action and may not have proper metabolic activation and detoxification
- the type and adversity of effects induced by the interaction of a chemical with endocrine function in animals need to be characterized and the relation of the endocrine dependent toxicity to the general toxicity profile of the chemical needs to be characterized
- absorption, distribution, metabolism and excretion have a very important role as determinants of biological activity of a chemical. These cannot be fully investigated *in-vitro* and cannot be predicted with sufficient reliability by PBPK-modelling without validation of the model using animal experiments (Gelbke *et al.* 2004; Gray *et al.* 2002).

Furthermore, the SCHER also points out the problem that the exclusive use of *in-vitro* tests for detection and assessment of “endocrine disruptors” may generate both false negative and false positive results. Negative results in *in-vitro* testing will result in the assumption that a toxic effect in animals or humans is absent, which may or may not be correct. The use of unrealistic concentrations of a chemical in *in-vitro* testing may results

in a conclusion of health hazards even when the high concentrations required to induce responses *in vitro* are highly unlikely to be reached *in vivo* due to poor absorption, or rapid biotransformation and excretion.

Based on these arguments, the SCHER is of the opinion that the *in vitro* approach proposed by BUAV-report will not predict possible endocrine system dependent toxicities in animals or in humans with the required reliability, and will not provide potency predictions nor data on dose-response for adverse effects. Since this information is essential, a conclusive risk assessment cannot be performed based the approach proposed by the BUAV-report.

It also needs to be recognized that modifications of the some of the present protocols for animal toxicity testing will already permit valid conclusions (Ashby 2003; Ashby *et al.* 2002). Inclusions of additional endpoints in study protocols for routine animal toxicity studies already required for classification and labelling and for hazard assessment (enhanced OECD TG 407, 28 day toxicity studies in rats) will also characterize effects of a chemical on the endocrine system. The available data on the performance of the enhanced OECD TG 407 indicate that this assay can reliably detect effects on the endocrine system, which are relevant for toxicity (Gelbke *et al.* 2004; Yamasaki *et al.* 2002). Scientists in Europe have been reluctant to include other animal testing approaches in the prioritization of chemicals for endocrine toxicity testing, i.e. the Hershberger assay and the uterotropic assay, in view of the additional animal experimentation (Kanno *et al.* 2003a, 2003b; Owens *et al.* 2003; Yamasaki *et al.* 2004) and the predictivity of the enhanced OECD 407 test. In addition, the OECD is reviewing and adapting the guidelines for ecotoxicity tests on fish, amphibians, invertebrates and birds to cover hormone mediated effects. Detailed reviews on the performance of the fish screening assay, amphibian metamorphosis assay and avian two-generation assay (OECD 2004; US-EPA 2002, 2003) are available. The adaptation of OECD TG 210 and 211 will be considered in the future.

These developments indicate that the current toxicity tests in animals, some of them specifically enhanced for endocrine parameters, are able to detect “endocrine disruptors” without requiring additional animal experimentation.

In contrast to the claims of the BUAV-report, animal toxicity testing has been shown to be highly predictive of human toxicities as detailed in the previous comments of the former CSTE (2004; (Olson *et al.* 2000). The variation among animal strains and species encountered in toxicity testing and pointed out as a problem in the BUAV-report is reminiscent of the variability in human and in animal populations and as such is always considered in risk assessments. The BUAV criticisms may be the consequence of a confusion of natural variability with uncertainty.

#### **4. RECOMMENDATION OF THE SCHER**

The *in-vitro* only testing approach by the BUAV-report will not provide reliable data as a basis for a science based risk assessment of exposures of chemicals with possible toxicities on the endocrine system. To minimize the use of animals in testing for toxicities to the endocrine system, the SCHER recommends:

- adaptation of a testing strategy as proposed by the US EPA and the OECD, which combines *in-vitro* testing and animal testing in a step-wise testing strategy, in Europe
- the testing strategy should include the modified protocols for routine toxicity studies to address effects on endocrine function in mammals and other species without additional animal experimentation

The SCHER also sees the need to clearly indicate that one of the major controversial issues in human health risk assessment of endocrine mediated toxicities is the possibility of low dose effects caused by "endocrine disruptors". While there is general agreement that high doses of potent hormones may cause a variety of toxicities in humans (Degen *et al.* 2002), some authors describe low-dose effects and non-monotonous dose-response curves with some estrogenic chemicals in mammals (Vom Saal and Hughes 2005; Vom Saal *et al.* 2005; Welshons *et al.* 2003). These findings could not be reproduced using toxicity testing following OECD-guidelines including low-dose exposure groups (Ashby *et al.* 1999; Cagen *et al.* 1999a, 1999b; Ema *et al.* 2001; Tinwell *et al.* 2002; Tyl *et al.* 2002). The assessment of such effects requires a rigorous and science based weight-of-the-evidence approach, which needs to consider that the findings at low doses represent changes without, or, at best, unknown toxicological significance. Moreover, the major differences in hormonal environments in rodent and human gestation (Witorsch 2002) and the frequent human exposure to a large number of naturally occurring chemicals with significant hormonal activity are to be included in the risk characterisation.

Some of the main concerns regarding environmental risk assessment of potential effects on the endocrine system in wildlife are the limited capacities of current test methods and endpoints for covering hormone related effects and the potential for long term effects associated with short exposures during critical periods of development. In this context, the SCHER also fully supports the activities of the OECD regarding revision and adaptation of test methods and recommends the development of a scientifically sound testing strategy for environmental effects of "endocrine-disrupting" chemicals to be included in the European Chemicals Policy.

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