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ECVAM European Centre for the Validation of Alternative Methods

STATEMENT ON THE SCIENTIFIC ACCEPTABILITY AND PRACTICAL AVAILABILITY OF *IN VITRO* METHODS FOR THEPRODUCTION OF MONOCLONAL ANTIBODIES

At its 10th meeting, held on 31 March 1998 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the ECVAM Scientific Advisory Committee (ESAC)[1] unanimously endorsed the following statement:

> For all levels of monoclonal antibody production, scientifically acceptable *in vitro* methods are now practicably available. These methods have been shown to be either better than, or equal to, the *in vivo* (ascites) production method in terms of antibody quality. Therefore, the *in vivo* production of monoclonal antibodies by the ascites method is no longer scientifically necessary, except in rare cases.

This endorsement was based on the conclusions and recommendations of ECVAM workshop report 23 on *Monoclonal Antibody Production*[2] and a report on monoclonal antibody (MAb) production which was produced for the ESAC.[3] The aim of both documents was to evaluate the present status of *in vitro* methods for MAb production in terms of: a) the antibody production capacity; and b) the concentration, yield and quality of the MAbs produced; and to compare the advantages and disadvantages of the *in vitro* methods with those of the traditional malignant ascites method. The evaluation showed that there are various scientifically satisfactory *in vitro* production systems available.[4]

The production of MAbs by the ascites method might be scientifically justifiable in the following rare cases: a) an exceptional need for an emergency therapeutic application; b) an existing regulatory approval for a diagnostic or therapeutic MAb produced *in vivo* (this has to be accepted until the approval expires); and c) in very exceptional circumstances where verifiable efforts have failed to produce the MAb *in vitro*.[5]

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1. The ESAC was established by the European Commission, and is composed of representatives of the EU Member States, industry, academia and animal welfare, together with representatives of the relevant Commission services. The following members of the ESAC were present at the meeting on 31 March 1998:

Dr B Blaauboer (ERGATT)
Professor J Castell (Spain)
Dr B Garthoff (EFPIA)
Dr C Hendriksen (The Netherlands)
Professor G Papadopoulos (Greece)
Dr B Rusche (Eurogroup for Animal Welfare)
Professor H Spielmann (Germany)
Professor H Tritthart (Austria)
Professor E Walum (Sweden)

Dr P Botham (ECETOC)
Dr D Clark (UK)
Professor A Guillouzo (France)
Dr R Lorenzini (Italy)
Professor V Rogiers (Belgium)
Dr O de Silva (COLIPA)
Dr O Svendsen (Denmark)
Dr M Viluksela (Finland)

Professor M Balls (ECVAM)
Dr J Fentem (ECVAM)
Ms S Louhimies (DGXI)
Mr A Van Elst (DGXXIV)

Mr G Corcelle (DGXI)
Dr G Fracchia (DGXII)
Dr M Robert (DGIII)

2. Marx U, Embleton MJ, Fischer R, Gruber FP, Hansson U, Heuer J, de Leeuw W, Logtenberg T, Merz W, Portetelle D, Romette J-L & Straughan D (1997) Monoclonal antibody production. The report and recommendations of ECVAM workshop 23. *ATLA* **25**, 121-137.

3. Dr Coenraad Hendriksen produced a report on the production of MAbs at the request of the ESAC, which will be published in *ATLA*.

4. Appendix 1 includes a list of reference publications on *in vitro* methods for the production of MAbs. In addition, a special issue of *Forum in Immunology* is to be published at the end of 1998, which will be dedicated to the *in vitro* production of MAbs.

5. Appendix 2 provides information on the regulation of MAb production in several EU Member States.

Appendix 1

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Appendix 2

Several EU Member States (Sweden, UK, Germany, The Netherlands) have already taken into account the availability and acceptability of *in vitro* methods for the production of MAbs, and have established guidelines on MAb production which restrict permission for the *in vivo* production of MAbs:

- *Sweden*: in May 1990, the Swedish National Board for Laboratory Animals issued a general recommendation regarding MAb production which stated that existing alternative methods should normally be used, but that use of the ascites method can be justified in certain cases, such as for the purification of infected hybridomas.[1]

- *United Kingdom*: in December 1991, the UK Home Office issued advice on protocols for minimal severity for raising antibodies using live animals.[2] According to this advice, "The malignant ascites method may be justified where less than 20 mice are needed on a one-off basis for a particular MAb. If appropriate facilities for the production of the MAbs *in vitro* are available, it is expected that these will be used in preference to the ascites method in mice."

  On November 6, 1997, the UK Home Office stated, under point 19 in the Supplementary Note of the Secretary of State's response to the Interim Report on the Review of the Operation of the *Animals (Scientific Procedures) Act 1986*, that "Exceptional justification will be needed in 12 months' time for use of ascitic mice in monoclonal antibody production.

New licences will not be issued unless *in vitro* attempts at production have failed or the use of animals is justified for specific diagnostic or therapeutic products."

- *Germany*: in accordance with the consensus achieved at a hearing held at ZEBET in 1989 (Berlin), the Federal Ministry of Food, Agriculture and Forestry stated that the *in vivo* production of MAbs is only allowed in the following exceptional circumstances: a) when the MAbs are intended for diagnostic or therapeutic emergency application in humans; b) when hybridoma cells need to be rescued because they have either failed to grow *in vitro* or they have become infected; and c) when the MAbs are needed to investigate new scientific problems.[3]

- *The Netherlands*: in January 1996, The Netherlands Veterinary Public Health Inspectorate stated in its official letter to the institutions concerned that *in vivo* production of MAbs is prohibited.[4] Exemptions are only granted on the basis of a good justification, taking into account the advice provided by a national expert committee. Prior to its prohibition, *in vivo* production of MAbs had already been regulated by The Netherlands Veterinary Public Health Inspectorate which, in 1989, issued a *Code of Practice for the Production of Monoclonal Antibodies* (e.g. restriction to 5-10 mice per hybridoma).[5]

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3. Anon (1993) *Tierschutzbericht 1993*. Drucksache 12/4242, pp. 49-50. Bonn: Bundesministerium für Ernährung, Landwirtschaft und Forsten.

4. Anon (1996) Official letter of the Veterinary Public Health Inspectorate. VHI/D/U-967/jw dated 5 January 1996.

5. Anon (1989) *Code of Practice for the Production of Monoclonal Antibodies,* 6 pp. Rijswijk, The Netherlands: Veterinary Public Health Inspectorate, Department of Animal Experimentation.