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NCad-2020-057

Date: August 24, 2020
Subject: Advisory letter on the EURL-ECVAM report on antibody production without the use of laboratory animals

Dear CCD members and chair,

EURL-ECVAM recently published its report entitled *Recommendation on non-animal-derived antibodies*, in which it recommends that – in the absence of solid scientific justification – animals should no longer be used for the development and production of antibodies for research, legally required research, diagnostics and therapeutic applications, and that EU Member States should no longer license the development and production of antibodies for these purposes, if these antibodies are acquired by immunising animals (with reference to EU Directive 2010/63/EU).

In response to this report, in early July, the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad: *Nationaal Comité advies dierproevenbeleid*) received the following request for advice from the Central Authority for Scientific Procedures on Animals (CCD: *Centrale Commissie Dierproeven*):

"For monoclonal antibodies, it now seems clear that the phage display technique in particular has been sufficiently developed to serve as a fully suitable alternative to antibody production, although perhaps not yet for multiclonal or polyclonal antibodies. On this point in particular, the CCD kindly requests the NCad to clarify the interpretation of the report and the consequences for the assessment of project licence applications in the Netherlands."

Situation in the Netherlands in brief
In the Netherlands, some 2000 animals are used each year for the production of antibodies (source: https://www.centralecommissiedierproeven.nl/documenten). The EURL-ECVAM report indicates that monoclonal antibody production using the ascites method should no longer be accepted under any circumstances (p. 9 of the report). This method, which causes severe harm to laboratory animals, is not used in any of the projects for which a licence has been issued in the Netherlands and

has not been used in the Netherlands for many years now. Ongoing projects concern immunising animals for the production of polyclonal antibodies and for the production of specific immune cells for monoclonal antibody production.
These immune cells are 'harvested' and then used for the *in vitro* production of monoclonal antibodies. These procedures cause mild to moderate harm to the animals. EURL-ECVAM's recommendation may have implications for any parties whose activities (research and/or production) rely on monoclonal and polyclonal antibodies.

Analysis

The EURL-ECVAM report concludes the following:
"Since phage display is a mature and proven non-animal technology for the development and production of reliable and relevant antibodies or affinity reagents, projects requesting authorisation for the use of animals for these purposes should systematically be challenged (in line with Articles 4 and 13 of Directive 2010/63/EU) and rejected by the authorising bodies where robust, legitimate scientific justification is lacking. In the light of the ESAC[1] Opinion (Annex 1) and the ESAC Working Group report (Annex 2), no scientifically justified exceptions could be identified." (p. 9 of the EURL-ECVAM report)
Nevertheless, the EURL-ECVAM recommendation leaves open the possibility that there may be 'sound scientific arguments' for departing from this recommendation. However, the ECVAM report does not provide them. Based on initial contacts and discussions with experts, the NCad concludes that opinions differ among the experts about whether laboratory animals can be completely replaced for (monoclonal) antibody development and production when using *in vitro* techniques such as the phage display technique, as stated in the ECVAM report:
"Based on the available scientific literature, application examples and the experts' own extensive experience, the ESAC concluded that non-animal-derived antibodies are mature reagents generated by a proven technology that are not only equivalent to animal derived antibodies, but in many respects can offer significant scientific advantages and economic benefits." (p. 5 of the EURL-ECVAM report)
For monoclonal antibodies, the various opinions are mainly focused on scientific arguments. In addition, economic and legal aspects can be complicating factors in transitioning to methods that do not use experimental animals.
For polyclonal antibodies, somewhat more general doubts exist concerning the current option of replacing them with the 'multiclonal' antibodies mentioned in the report, consisting of a mixture of monoclonal antibodies. There is currently insufficient scientific literature to support the EURL-ECVAM recommendation to replace polyclonal antibodies with multiclonal antibodies.

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1 ESAC: ECVAM Scientific Advisory Committee

Advice

The NCad endorses the need for solid scientific justification when applying for a project licence to use experimental animals, as is standard procedure in the Netherlands. The EU Directive requires the use of methods that do not involve the use of experimental animals, if such methods give equivalent or better results. However, the NCad notes that opinions differ in the literature and among experts on the general applicability of methods that do not involve the use of experimental animals for the development and production of monoclonal and polyclonal antibodies. A complete ban on the use of experimental animals for antibody production therefore currently seems premature to the NCad. The NCad advises the CCD to be prudent in its assessment of project licence applications in which the production of antibodies using experimental animals play a role.

We recommend the following working method for the assessment:

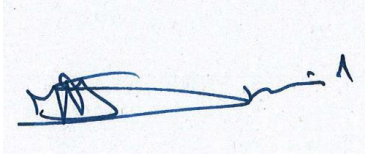
1. The applicant must provide a detailed justification as to why laboratory animals are required for the production of the antibodies.
    a. In the case of polyclonal antibody production, the following questions must be answered:
        - Why are monoclonal antibodies not being used?
        - Why are multiclonal antibodies not being used?

    b. In the case of monoclonal antibody production, the following question must be answered:
        - Why are techniques not being used that do not require the use of experimental animals, such as the phage display technology?

2. If it is deemed necessary, the CCD shall have the justification provided being reviewed by one or more independent *experts in the field*. At the request of the CCD, the NCad can provide names of experts who can be consulted.

The NCad will continue to closely monitor developments related to antibody production. To this end, the NCad will consult experts and monitor the activities in other EU Member States. The NCad will inform the CCD of its findings.

Kind regards,



Henk Smid , Chair of the Netherlands National Committee for the Protection of Animals Used for Scientific Purposes