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Dear Dr. Stokes:

These comments are submitted on behalf of the Alternatives Research & Development Foundation (ARDF), the American Anti-Vivisection Society (AAVS), People for the Ethical Treatment of Animals (PETA), and the Physicians Committee for Responsible Medicine (PCRM)—a coalition organizations dedicated to animal protection, development of alternative methods, and health advocacy representing more than 1.2 million Americans. We are submitting these comments in response to an October 16, 2006 notice in the *Federal Register* inviting public comment on the NICEATM Pre-Screen Evaluation of a Test Method Nomination: MCF-7 Cell Proliferation Assay to Detect Estrogenic Activity (the CCi test method). We consider this method to have great potential for reducing the number of animals used in screening for endocrine disrupting activity.

An international peer review of this cell proliferation assay of estrogenic activity is appropriate, necessary, and should be accorded high priority. A thorough yet expeditious review by an expert panel resulting in the endorsement of this test method as a Tier 1 screen is a crucial step forward in the efforts of ICCVAM to develop a tiered endocrine disruptor screening program and to meet its statutory mandate to promote the replacement, reduction, and refinement of animal-based testing (42 *U.S.C.* Sec. 2851-3(b)).

The need for* in vitro *test methods for screening potential endocrine disruptors

We agree that the CCi test method would address the need identified by the US EPA's former Endocrine Disruptor Steering and Testing Advisory Committee for an automated, high-throughput Tier 1 assay to identify substances that have the potential to interact with the endocrine system. Validation of any assay fulfilling this need is of immediate concern. The proposed method is advantageous in that it can identify potential endocrine disrupting compounds in complex mixtures as well as purified chemicals and is amenable to high throughput screening. However, careful consideration should be given to whether this assay provides sufficient "value added" over existing transcriptional activation assays -which have already undergone rigorous inter-laboratory/international validation and are about to commence peer review - to warrant the resources required for full validation, especially when there are crucial endpoints (e.g. effects on the thyroid) for which there are currently no *in vitro* methods are in development.

In the past, there has been some hesitancy on the part of both the EPA and ICCVAM to develop transcriptional activation assays that use cell proliferation as the measured endpoint. The

ICCVAM pre-validation study appears to accept that the agonist (ICI 182,780) control measure incorporated into the CCi assay is sufficient to prove ER binding specificity. It is crucial to determine - before proceeding with a validation study - whether the regulatory agencies responsible for endocrine disruptor screening (primarily the EPA) will agree that the assay will have regulatory applicability once validated.

Extent to which the CertiChem Background Review Document (BRD) provides the information requested, adheres to the recommendations and shows adequate performance with regard to the ICCVAM Guidelines (Sections 2.2 – 2.4 of the Evaluation)

Since the BRD was not available, is not possible to assess the conclusions of the Review. This lack of transparency must be remedied for future meaningful review, as stipulated in ICCVAM's *Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (Appendix D: ICCVAM Validation and Regulatory Acceptance Criteria): "All data supporting the assessment of the validity of the test method must be available for review."

Of particular concern to us is Section 2.2.10, Assessment of Animal Welfare Considerations, which is merely labeled "complete." There are several additional points that deserve attention in future studies:

1) In section 2.2.2, it is noted that a rationale for the selected number of replicate samples per study or the number of repeat experiments was not provided. This issue needs to be clarified to assure statistical validation.

2) An issue common to the validation process of all endocrine assays is that there is "no accepted animal or human data set to serve as a reference for determining accuracy…"(Section 2.2.6). It is critical that human data be collected for comparison and that *in vitro* methods not validated by using data from animal studies.

3) Two comments in the Evaluation suggest that some standardization of methodology is needed prior to further validation (namely, a comment in Section 2.2.5 that suggested different protocols were used, with an explanation of the differences provided; and another comment stating there was no information regarding variability across plates vs. variability between wells on a single plate [footnote 1, page 10]).

In conclusion

The parties to this submission are strongly committed to advancing the development, validation, and regulatory acceptance of test methods that will reduce, and ultimately replace, the use of animals in toxicity testing. To support that goal, we encourage the validation of *in vitro* methods for identifying potential endocrine disrupting chemicals, as these methods will greatly reduce the number of animals subjected to equivocal *in vivo* tests such as the Uterotrophic and Hershberger assays. Specifically, given the caveats expressed above, we support further validation of the MCF-7 Cell Proliferation Assay, in addition to the pursuit of additional *in vitro* endocrine and androgen receptor binding and transcriptional activation assays.

Thank you for your consideration of these comments.

Sincerely,

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