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***Sent via e-mail to* whiteld@niehs.nih.gov**

Dear Dr. White:

The following comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM) in response to the July 14, 2014 Federal Register announcement by the National Institutes of Health (NIH), "Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments."

Update on ICCVAM Activities

1. New Vision and Direction for ICCVAM
   As we have commented previously, we are extremely pleased with ICCVAM's new direction and its much stronger focus on achieving real reductions in animal use. The appointment of Dr. Warren Casey as the Director of NICEATM was a welcome change, and we have had very positive interactions with Dr. Casey over the past two years.

   With respect to the suggestion that activity updates be regularly communicated through a web-based approach, we believe these updates would be very helpful, particularly if both agency-specific and NICEATM's own activities related to implementing the 3Rs could be posted on the same website.

2. ICCVAM Biennial Report 2012-2013
   We found this report to be an excellent summary of activities over the past two years and are pleased to see the more proactive roles that ICCVAM and NICEATM are taking to foster replacement and reduction of animal use.

   a. We encourage ICCVAM member agencies to consider and communicate how they might use *in vitro* hepatic metabolism methods evaluated by the European Union Reference Laboratory for the Validation of Alternative Methods (EURL ECVAM) (pg. 20).

b. We recognize the value of building high-quality reference data libraries for the evaluation of new methods and the building of pathway toxicology knowledge. We are particularly interested in respiratory sensitization data and would welcome collaboration, as PCRM is leading an adverse outcome pathway (AOP) project for this endpoint. Likewise, inhalation and oral toxicity data will be useful for the working groups with which PETA is involved.

c. Endocrine disruption activities: We encourage NICEATM and ICCVAM member agencies to consider how they might contribute to the development of particular assays to detect thyroid disruption that are near maturity and outlined in the OECD document No. 207: "New Scoping Document on *in vitro* and *ex vivo* Assays for the Identification of Modulators of Thyroid Hormone Signalling."[1]

d. We are pleased to see the work that the Department of the Interior is doing to reduce the testing of fish and birds. We encourage OPP and OPPTS at the EPA to collaborate and bring on board some of those strategies, if possible.

3. Acute Oral and Dermal Toxicity Testing

It appears that significant progress has been made in creating the draft dataset of acute oral and dermal LD_{50} data, the analysis of which will hopefully lead to the eventual elimination of one of the tests. The Competent Authorities for REACH and CLP (CARACAL) have recently agreed on proposals to amend REACH Annex VIII to this end for unclassified chemicals.[2]

We look forward to the day when acute testing can be eliminated completely from hazard testing requirements and replaced with a complement of *in silico* and *in vitro* assays. As we noted last year, EURL ECVAM's 2013 recommendation on the 3T3 NRU assay[3] should be considered a first step in distinguishing potentially toxic substances from nontoxic ones. This method was found to have a low false negative rate and was particularly relevant to the assessment of industrial chemicals and other substances not designed to act on specific biological targets. EURL ECVAM has since drafted a strategy to avoid, reduce, and refine the use of animals in assessing acute systemic toxicity. The use of repeated dose toxicity data to support classification and labeling for acute systemic toxicity is among the objectives discussed in this strategy; available repeated dose information should be added to the dataset.

EURL ECVAM's strategy also recommended organizing mechanistic knowledge about acute oral toxicity (for example through the use of AOPs) and making improvements to the 3T3 NRU assay. Finally, in their 2013 report on results from the European Project ACuteTox, Prieto *et al.* noted that complementing the 3T3 NRU with other *in vitro* assays did not

[1] OECD. (2014) Series on Testing and Assessment No 207: New Scoping Document on *in vitro* and *ex vivo* Assays for the Identification of Modulators of Thyroid Hormone Signalling. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2014)23&doclanguage=en. Accessed 28 August 2014.

[2] Chemical Watch. 17 July 2014. Caracal agrees on need to drop acute dermal toxicity tests.

[3] EURL ECVAM Recommendation on the 3T3 NRU Assay for Supporting the Identification of Substances Not Requiring Classification for Acute Oral Toxicity. Available at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/3t3-nru-recommendation. Accessed 29 August 2014.

significantly improve the classification of compounds, possibly due to the fact that the currently applied classification systems are based on arbitrary cut-off values for rat oral LD_{50}. A revision of the current classification schemes to be more mechanistically based was recommended.[4] A classification system or set of classification schemes with chemical rankings within common modes of action may be more relevant to human health protection and be more amenable to the use of *in vitro* assays. We encourage ICCVAM to continue to focus on acute toxicity as a priority area and to continue to work closely with EURL ECVAM on this effort.

4. Skin Sensitization Testing
   a. A number of nonanimal test methods are now available for evaluating this endpoint. As mentioned in ICCVAM's update, the AOP for skin sensitization is well characterized and OECD test guidelines have been drafted for the KeratinoSens, DPRA, and h-CLAT assays. Sufficient data now exist for developing a reliable testing strategy that avoids the use of animals and should be finalized without delay. In addition to the database that ICCVAM is curating, various pesticide industry companies possess data and experience with these methods. We are working with these companies and CropLife America to set up a database and compare results of *in vitro* tests with *in vivo* tests, and we encourage NICEATM and EPA to stay involved in this effort.
   b. ICCVAM member agencies and NICEATM have done much on this endpoint in recent years and we look forward to hearing more about it at the SACATM meeting. We also encourage specific outreach to CPSC and FDA-regulated industries (where relevant) to ensure that regulatory and industry needs align to replace the GPMT and LLNA as quickly as possible.

5. Vaccine Potency and Safety Testing

   The organization and support of workshops focusing on animals used in biologics testing has been crucial to the development of policies that have facilitated industry adoption of nonanimal methods as well as refinement and reduction strategies in areas where replacement methods are not yet approved. We strongly encourage NICEATM and ICCVAM to expand their efforts in this area to ensure that roadblocks and promising solutions identified at previous events receive the long-term evaluation and development that will be required for large scale successes. We look forward to regular updates on progress related to the objectives outlined in these workshops, especially with regard to *Leptospira* and rabies vaccine potency test refinement and replacement options.

6. Additional scientific areas that ICCVAM and NICEATM should consider pursuing
   a. **Fish Embryo Toxicity (FET) Test**. Fish embryo-based methods have been proposed for a number of years as an alternative to the acute fish toxicity test performed with juvenile or adult fish. The acute test is used in both whole effluent monitoring and as a component of eco-toxicity evaluation of pesticides and other chemicals. EURL ECVAM finalized its

[4] Prieto P, Kinsner-Ovaskainen, A, Stanzel S, et al. (2013) The value of selected *in vitro* and *in silico* methods to predict acute oral toxicity in a regulatory context: Results from the European Project ACuteTox. Toxicology in Vitro **27**(4):357-376.

recommendation on the use of the FET test in July 2014.[5] The recommendation states that this test "is suitable for predicting effects in species other than zebrafish and can provide information on acute fish toxicity comparable to that of the standard test (OECD TG203)." The recommendation goes on to note that, now that the FET is fully validated and is available as a standardized OECD test guideline (TG236),[6] it can be used as an alternative to acute fish toxicity testing under the EU REACH program and where appropriate should be used for generating information on this endpoint. The FET has been used routinely as a regulatory test in Germany for whole effluent monitoring for a number of years.

We have contacted the EPA in writing regarding acceptance of this method in place of OECD TG203 (also OPPTS 850.1075) for both whole effluent testing and chemical hazard evaluation. In their response to us, the Water Permits Program of EPA appears open to considering the FET, however the agency is not at the point of accepting the test as a one-for-one replacement for TG203 at this time. We urge ICCVAM/NICEATM to work with the EPA to implement the method as appropriate.

b. **Update on Communication Efforts and Report on Outcome of ICCVAM Public Forum**. We would like to compliment ICCVAM and /NICEATM on their improved communication, outreach efforts, and increased transparency. We have found NICEATM Director Warren Casey and ICCVAM co-chair Anna Lowit to be especially accessible and open to new ideas and directions.

We appreciate the effort ICCVAM has invested in coordinating workshops, working groups, webinars, and other events that bring together industry, regulators, and affiliated organizations. These events have proven crucial in advancing the development and implementation of alternative testing strategies, including the "Collaborative Workshop on Aquatic Models and 21st Century Toxicology," the "Workshop on Alternatives to the HIST for Acellular Pertussis Vaccines," the "Adverse Outcome Pathways: From Research to Regulation" workshop, and the biologics workshops mentioned above. We encourage ICCVAM to continue this essential work, and we look forward to participating and contributing to these meetings in the future. We applaud the decision to involve stakeholders at the beginning of new efforts as well as in annual planned opportunities to engage the public. We found June's stakeholder forum to be very useful, and we hope it was useful to ICCVAM and NICEATM as well.

As work continues in this area, we encourage ICCVAM to ensure that its focus on increasing awareness of 3Rs opportunities includes a routine approach to revising its test method recommendations to member agencies. As approved methods are optimized over time through expansion of applicability domains and other technical capacities,

[5] EURL ECVAM Recommendation on the Zebrafish Embryo Acute Toxicity Test Method (ZFET) for Acute Aquatic Toxicity Testing. Available at: http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/zfet-recommendation. Accessed 29 August 2014.

[6] OECD. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. Available at: http://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en. Accessed 29 August 2014.

ICCVAM's recommendations and agencies' responses to those recommendations should likewise evolve. We encourage ICCVAM to ensure that all test method evaluations are accompanied by up-to-date recommendations and agency response letters, even when these methods have been published as OECD test guidelines. The ICCVAM test method recommendations for EPISKIN, EpiDerm, and the Rat Skin Transcutaneous Electrical Resistance Assay, for instance, have not been updated since the 2002 test method evaluation report, and none of the transmittal letters are available on the ICCVAM website.[7] Much has changed in the interim—including the content of the referenced OECD test guidelines—and agency perspectives on their applicability should be revised and made public.

We also encourage NICEATM and ICCVAM to consider how methods for which no ICCVAM evaluation or recommendation to date has been made, but which are considered acceptable by other international validation agencies and/or are OECD test guidelines, might be integrated into agencies' regulatory programs, and how recommendations can be made for these methods.

c. **Update on International Collaborations/ICATM and Interactions with EURL ECVAM**. We are pleased to see that ICCVAM and NICEATM are committed to working closely with EURL ECVAM on developing new alternative test methods. Ensuring that scientists in the U.S. are actively participating in the entire test method evaluation process will hopefully eliminate the duplicative and lengthy reviews that have occurred in the past.

While ICCVAM is a member of the International Cooperation on Alternative Test Methods (ICATM), this group could be much more effective at fostering the harmonization of methods amongst its member countries. For example, ICATM has not been effective with regard to the issue of the 1-year dog toxicity study for pesticide registration. The EPA eliminated the requirement for this test in 2007 (CFR 158.500, 26. Oct 2007, p 60976) after finding that the data generated did not add value to risk assessment beyond the results of the already required 90-day dog study.[8,9,10] The EU has also lifted this requirement, but both Canada and Japan still have provisions for conducting a 1-year dog study. This area is one area where ICCVAM could use its membership in ICATM to spearhead a much-needed change.

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[7] Available at: http://ntp.niehs.nih.gov/iccvam/docs/dermal_docs/cwgfinal02/06_sec1.pdf. Accessed 29 August 2014.

[8] Dellarco VL, Rowland J, May B. (2010) A retrospective analysis of toxicity studies in dogs and impact on the chronic reference dose for conventional pesticide chemicals. Crit Rev Toxicol 40(1):16-23.

[9] USEPA. (2006) Length of Dog Toxicity Study(ies) that is Appropriate for Chronic RfD Determinations of Pesticide Chemicals. Washington, DC: Health Effects Division, Office of Pesticide Programs, US Environmental Protection Agency. March 20, 2006. Available at: www.regulations.gov (Docket # EPA-HQ-OPP-2004-0387-0179). Accessed 29 August 2014.

[10] Baetcke KP, Phang W, Dellarco V. (2005) A Comparison of the Results of Studies on Pesticides from 12- or 24-Month Dog Studies with Shorter Duration. US EPA, 4/7/05. Available at: http://www.epa.gov/scipoly/sap/meetings/2005/may2/dogstudymay05.pdf. Accessed 29 August 2014.

Similar problems arise from the lack of collaboration with all relevant authorities in the harmonization process, including the US Pharmacopeia (USP) and the International Organization for Standardization (ISO). As a consequence, guidance from the USP, ISO, ICCVAM member agencies, and other regulators can differ on the acceptability of a given nonanimal test method. A series of collaborative studies in 1994, for instance, led to the replacement of *in vivo* somatropin batch release bioassays in the European Pharmacopoeia (Ph. Eur.) with a physicochemical liquid chromatography assay.[11,12,13] Our correspondence on this matter with the USP and the FDA failed to clarify the rationale for retaining the *in vivo* assays that had been abandoned in the EU. The same issue exists for other recombinant hormones, including insulin, despite the USP's ongoing efforts to develop cell-based assays to use in place of the current pharmacopeial standards. As with guidance prepared by the USP, the absence of ISO from ICATM and ICCVAM discussions has ensured that regulated industries approach newly approved methods conservatively, if at all. Although EURL ECVAM's participation in ICATM ensures that progress achieved in integrating nonanimal methods into Ph. Eur. is discussed by ICCVAM member agencies, these agencies do not have a similar direct relationship with the USP or ISO. Without integrating the USP and ISO into harmonization discussions through ICATM or ICCVAM, implementation of validated, approved, nonanimal methods will continue to stagnate.

Thank you for the opportunity to comment. We look forward to continued productive collaborations in the future.

Sincerely,

[Redacted]

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[11] Bayol A. et al. (2004) Somatropin and its variants: structural characterization and methods of analysis. Pharmeuropa Bio 1:35-39.
[12] Bristow A, Jeffcoate S. (1992) Analysis of therapeutic growth hormone preparations: report of an interlaboratory collaborative study on growth hormone assay methodologies. Biologicals 20:221-231.
[13] EDQM. (2008) *Monograph 0951: somatropin*. European Pharmacopoeia 7.0, 2954-2956. Strasbourg, Council of Europe.