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Dear Dr. Stokes:

We are pleased to submit comments in response to the National Institute of Environmental Health Sciences' (NIEHS) *Federal Register* notice dated September 28, 2001, inviting public comment on the "Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity" and the associated "Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity." These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and our 750,000 members and supporters who are concerned about the use of animals in cruel and non-validated lethal poisoning tests.

We appreciate the effort needed to organize this workshop, which followed the October 1999 agreement between the White House and the animal protection community. That agreement set forth minimal animal protection measures to be taken in the Environmental Protection Agency's (EPA) high production volume (HPV) chemical-testing program, including further research into the use of *in vitro* cytotoxicity assays as a replacement for lethal dose tests and their prompt incorporation into the HPV program. We also greatly appreciate the work of such forward-thinking individuals as Drs. Liebsch, Fentem, and Curren to advance the use of the cytotoxicity assays in regulatory testing. However, a number of aspects of the workshop report and guidance document are troubling, most notable of which is the apparent lack of progress on the cytotoxicity test as a replacement method.

A disproportionate amount of time and attention has been focused on refining existing animal tests, to the detriment of efforts to fully replace the use of animals in acute toxicity testing. As you are no doubt aware, studies by ZEBET in Germany have demonstrated that, although the number of animals used and the number of deaths seen in the Up-and-Down Procedure, Acute Toxic Class Method, and Fixed Dose Procedure could possibly be further reduced, such a reduction would be modest at best. Therefore, current efforts to use *in vitro* data solely for the purpose of calculating an *in vivo* starting dose miss the mark by focusing on an excessively narrow, limited, and ultimately restrictive application of an enormously promising methodology.

We are also extremely concerned about the apparent lack of a framework or mechanism for the collection and dissemination of data from *in vitro* cytotoxicity assays used in starting dose calculations, given the importance of these data to the pre-validation and validation of these assays for use as a replacement method. Such a framework should be developed for immediate implementation by federal agencies and all prospective end-users of these test methods in order to ensure that sufficient quantities of appropriate data are available for future use. In particular, the EPA must immediately incorporate these *in vitro* cytotoxicity assays into its HPV chemical-testing program and provide a framework to all participants so that the validation of these tests as a replacement method may proceed with all due speed. There is simply no excuse for this data being wasted by not having the framework in place from the beginning.

The workshop report specifically notes that the use of *in vitro* data to estimate *in vivo* starting doses should occur as a "parallel activity" (p. 31). However, efforts to date have been limited to reduction and refinement initiatives, with little or no attention being paid to what was clearly intended to be a "parallel" path toward the use of *in vitro* cytotoxicity assays as a total replacement method.

Workshop participants recommended that, in order to further the goal of replacing the use of animals in acute lethality assays, "a working group of scientific experts should be established to identify and/or define specific *in vitro* cytotoxicity test protocols for inclusion in a prevalidation study of their use for predicting LD50 values" (p. xxiii) and that such a prevalidation study "should be initiated ***as soon as possible***…" (p. xxii, emphasis added). However, in more than a year since the workshop was convened, there has been no evidence of forward movement on designing or implementing a pre-validation study, or even establishing a working group of scientific experts to provide advice in this regard. Nor has there been any apparent effort to advance key research priorities identified in the workshop report. For example, participants recommended that:

- "The MEIC human database should be peer-reviewed, modified if needed, and expanded ***as soon as possible*** so that data will be available for future validation studies." (p. xxiii, emphasis added)
- "[A]n up-to-date review of current QSAR systems for predicting rodent oral LD-50 values should be undertaken." (p. xxiii)
- "Continued development and optimization of [simple systems that predict gut absorption, BBB passage, key kinetic parameters and metabolism] is encouraged and should receive regulatory support." (p. xxiii)
- "A worldwide database is needed to compare human *in vitro* and *in vivo* data for hepatic toxicity." (p. xxvi)
- "[G]eneral endpoints for *in vitro* neurotoxicity have been studied and used extensively and are ready for formal validation." (p. xxvi)

In October 2000, PETA suggested a number of steps that could be taken immediately by the EPA and/or NIEHS in order to facilitate validation of cytotoxicity assays as a replacement method. These included the immediate establishment of a management team/working group to design the pre-validation study for the *in vitro* replacement tests. Because of European predominance and a dearth of U.S. scientists with expertise in this arena, we recommended that international scientists play an important role in this process. We also suggested that chemical selection and database construction begin immediately and that human data be collected and compiled so that such data are

ready for immediate use in the validation process. As far as we know, more than a year later, none of these suggestions has yet been implemented.

It must be emphasized that workshop participants "considered that, ***if the commitment to conducting a formal validation study was strong enough***, the scientific resources could be harnessed for this effort with facility and the *in vitro* tests studied proved good enough, ***a replacement test battery might be achieved in as short a time as 2-3 years***" (p. 31, emphasis added). It is sobering indeed to realize that, far from being one-third of the way toward replacing the use of animals in acute toxicity testing, regulatory agencies are only now beginning to consider the use of *in vitro* cytotoxicity assays even to predict an *in vivo* starting dose. This lack of progress is unacceptable and reflects a glaring lack of commitment by regulatory authorities to the replacement of lethal animal poisoning tests.

As recommended by the workshop participants, we urge ICCVAM and its partners worldwide to begin an immediate and coordinated effort to validate the use of *in vitro* cytotoxicity assays as a replacement method for acute toxicity testing *in vivo*. We realize that this suggestion is neither new, nor necessarily popular, with some in the toxicological, regulatory, and regulated communities. However, whatever resistance exists must no longer be allowed to act as a barrier to *in vitro* methods achieving their full potential as accepted regulatory tests for acute toxicity.

Those who object to the use of *in vitro* methods as replacement methods on the grounds that they have not been "adequately validated" should be reminded that the three current LD-50 refinement methods have only been assessed relative to rodent LD-50 data which, in itself, has never been validated. As Dr. Leon Bruner of Gillette stated at the conclusion of the workshop, scientists are even uncertain as to whether the rat LD-50 test is able to predict lethality in rats, let alone in humans. A formal validation study consistent with internationally accepted criteria has never been undertaken to determine the relevance of any rodent acute toxicity data to humans (or, for that matter, the relevance to humans of any animal-based test method). However, what comparative data do exist do not inspire a great deal of confidence in the status quo. For example, the MEIC study examined the results of rat and mouse acute toxicity tests for 50 chemicals and found that these tests were able to predict toxicity in humans with, at best, 65 percent accuracy. In contrast, a series of four *in vitro* human cell line tests was found to predict chemical toxicity in humans with up to 83 percent accuracy. This study clearly demonstrates the value of *in vitro* methods over non-validated animal tests in terms of their predictive accuracy alone, to say nothing of savings in time and costs — both financial and in terms of animal suffering and death. It is unconscionable that years have gone by since the results of the MEIC study were published, yet government agencies worldwide have made little or no effort to advance this groundbreaking work.

Lastly, we are disappointed with the relative overemphasis that has been placed on the use of non-human versus human-derived cells in the guidance document. The workshop report correctly states that "the ultimate goal is to be able to predict acute toxicity <u>in humans</u>." We therefore question the extent to which both ICCVAM's recommendations to agencies, and the guidance document itself, promote the use of a non-human (mouse fibroblast BALB/c 3T3) cell line over human (NHK) cells, and their failure to insist that all test methods be validated against human data, rather than against the results of non-validated animal tests. It is inappropriate to continue to give preferential treatment to non-human tissues and models over their human equivalents. A fundamental shift in priorities is

necessary in order to achieve the goal of developing "a battery of *in vitro* tests employing human cells…to predict human acute toxicity" (p. xxii).

The replacement of lethal poisoning studies with *in vitro* cytotoxicity assays is literally a life and death issue for animals as well as an issue of major importance to the animal protection community. It is therefore imperative that federal regulatory authorities, both in the United States and globally, do everything in their power to ensure the timely development, validation, and acceptance of *in vitro* cytotoxicity assays (utilizing human cells) for the evaluation of acute toxicity.

Thank you for your attention to these comments.

Sincerely,

J. Y. Sandler

Jessica Sandler, MHS
Federal Agency Liaison