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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

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Via electronic transmission to: niceatm@niehs.nih.gov

Dear Dr. Stokes:

These comments are submitted on behalf of People for the Ethical Treatment of Animals and our more than 1 million members and supporters in response to a January 27, 2006 notice in the *Federal Register* inviting public comment on the appropriateness and relative priority of convening a workshop addressing replacement of the mouse lethal dose 50 percent (LD50) test for botulinum neurotoxin (BoNT) potency testing. PETA supports this Humane Society of the United States (HSUS) nomination and agrees that this activity is important and appropriate and that the replacement of lethal BoNT potency tests in animals should be urgently pursued[1].

Lot release testing of biologicals consumes 10-20% of all animals used in laboratories and should be a high priority area for replacement efforts. The lot release testing of BoNT products presents an opportunity to replace a great deal of ongoing and readily avoidable animal testing. The HSUS nomination highlights the fact that an extremely cruel and outdated test (involving death by paralysis-related suffocation) is currently conducted on mice despite the fact that mechanistic human biology-based *in vitro* tests exist. The replacement of the BoNT LD50 by alternative tests could and should have happened years ago as it is both feasible and compelling. ICCVAM should build upon the momentum of the considerable efforts already expended towards this goal, and expeditiously work towards validation of the SNAP-25 assay and other *in vitro* tests.

A common barrier to *in vitro* test method development is a lack of mechanistic understanding, leading to the traditional reliance on experiments based on an unvalidated assumption of animal

[1] It should be noted that BoNT potency testing is most relevant to the U.S. Food and Drug Administration (FDA) and that HSUS apparently tried unsuccessfully to engage the FDA on this issue prior to submission to ICCVAM. ICCVAM's mandate is to provide a forum for replacing those tests which are most commonly required or used across many Agencies. However, in the absence of individual Agencies having their own formal and transparent mechanisms for validation of novel methods, increasingly, Agency-specific test methods are landing on ICCVAM's doorstep as the only means of achieving official validation in the US (of the last three ICCVAM submissions or nominations, two are mainly FDA-specific and one is mainly EPA-specific). To address this problem, Agencies should develop processes for Agency-specific method validation enabling ICCVAM to more proactively address ubiquitous and challenging endpoints such as carcinogenicity, developmental toxicity, and target organ toxicities, efforts towards which have stagnated. However, given the current lack of Agency-specific validation procedures, the current dearth of non-Agency-specific ICCVAM nominations/submissions, and the urgent imperative to replace the BoNT LD50 potency test with an available *in vitro* test, we support the use of the ICCVAM forum for this activity.

surrogacy. However, in this case, the mechanism by which BoNT leads to its paralytic and poisonous effects (inhibiting acetylcholine release through the cleavage of vesicle targeting proteins in pre-synaptic neurons) has been well studied and the relevant proteins and interactions have been thoroughly characterized. This enabled the development of functional *in vitro* assays (first published a decade ago) based on assessing the cleavage by BoNT proteins of human target peptides. One of these *in vitro* tests (the SNAP-25 assay) is already routinely used by the UK national control agency (NISBC). These well-established mechanistic human biology-based *in vitro* tests represent the best possible means of assessing BoNT product potency.

Thus, the proposed workshop should focus on how to most rapidly achieve the validation, adoption, and regulatory acceptance of the available *in vitro* tests as they give every indication of having excellent specificity, sensitivity, and speed but have not been the subject of a formal validation effort. The SNAP-25 assay should be validated for use as a standalone test wherever possible but even if is not deemed a complete replacement, this should not hold up efforts to validate and adopt it for the conditions for which it is appropriate. If there are any circumstances under which a follow-up test is necessary or the use of molecular *in vitro* tests may not be appropriate, a cell-based assay (or at worst, an *ex vivo* test) should be validated as an alternative. However, it is important not to create a system in which the majority of *in vitro* tests are followed up with another assay, especially one *in vivo* or *ex vivo*. While the alternative *in vivo* and *ex vivo* mouse assays described in the HSUS nomination involve protocols that are more clinically relevant and humane than the LD50, they should not be the focus of the proposed workshop when excellent *in vitro* tests are available. **The proposed ICCVAM workshop should strongly prioritize realizing validation of *in vitro* tests over the *in vivo* or *ex vivo* assays as the *in vitro* tests do not involve the use of animals and are likely to be more sensitive, specific, human-relevant, consistent, cost-effective, and quicker.**

As *in vitro* BoNT potency tests are assessed and especially as validation efforts are planned, it is crucial that the highly variable, less sensitive, and unvalidated mouse LD50 assay not be viewed as the gold standard. Other problems with the mouse LD50 assay include the fact that there are numerous biological differences between mice and humans which would clearly affect the nature of a mouse vs. human response to BoNT sample exposure and that it does not assess a clinically relevant endpoint (death instead of local paralysis). Thus, results from mechanistic human biology-based *in vitro* tests may not correlate with those from mouse LD50 assays. **Using LD50 results as reference data may make it more challenging or impossible to validate the *in vitro* tests.** The accuracy of validation efforts is always highest when using reference data relevant to the species of interest and biological endpoint. When human data is available or could be safely generated (e.g., through human foot method described in HSUS nomination), it should always be utilized as reference data. If this is not possible, a production consistency approach could be taken: The *in vitro* test could be run alongside the current method for a set number of batches in order to prove that its overall potency prediction aligns. **Regardless of what reference data is utilized, in no case should animals be subjected to LD50 tests solely to obtain reference data for a validation exercise.**

A few words of caution regarding the practicalities of the proposed ICCVAM workshop: The focus should be on prioritizing the potential tests and identifying the *most expedient means* of achieving their validation. Although this workshop would constitute ICCVAM's first official effort to address this issue, much groundwork has already been laid so the process should be hastened to the greatest extent possible. The SNAP-25 assay in particular is a test based on the relevant human mechanism and has been proven to work well, and it need not take years of prolonged study, additional meetings, and new studies in order to validate it and start using it for appropriate applications. If at all possible, it should be quickly and cost-effectively validated based on retrospective data (especially if data can be obtained from the NIBSC who has used this test for years). Another concern is that the HSUS nomination mentions several types of BoNT-related tests (BoNT product tests, antitoxin tests, diagnostic tests, and so on) which have related but distinct protocols. PETA recommends that the workshop address only BoNT product potency testing in order to provide focus to the effort; once clearly validated for one use, *in vitro* BoNT-related tests can more easily be adapted and validated for other uses. In any case, it is important that the scope of the workshop be clearly defined. Lastly, any ICCVAM efforts on this topic should of course continue to be closely coordinated with ongoing or imminent efforts in Europe.

In conclusion, the submitted nomination represents an opportunity to conduct an expeditious review and work towards rapidly replacing an outdated animal test with improved alternatives. We strongly urge ICCVAM to move ahead quickly to convene a panel of experts who can make the necessary scientific judgments regarding the proposed alternative tests with a view towards a speedy affirmation of their value in assessing BoNT potency. Consumer safety, scientific rigor, and animal welfare concerns will all be best served by promoting the use of human-relevant mechanistic *in vitro* assays for botulinum toxin related testing.

Thank you for your attention and responsiveness to these comments.

Sincerely,

Sadhana Dhruvakumar
Director, Medical Testing Issues
People for the Ethical Treatment of Animals