



Citizens for Alternatives to Animals Research & Experimentation

PO Box 102 • Ardsley, NY 10502 • 914-674-0577 • www.caareusa.org

September 14, 2016

These comments are submitted by Citizens for Alternatives to Animal Research & Experimentation (CAARE) in response to the *Request for Public Comment on Developing a U.S. Strategy and Roadmap for Implementing 21st Century Toxicity Testing Approaches*

CAARE is dedicated to promoting the use of scientifically superior, human-based research methods in place of animal tests and experiments.

CAARE appreciates the efforts by ICCVAM and NIEHS to address this issue. As well, we very much appreciate the efforts of NICEATM's Director, Dr. Warren Casey, to break ground where this issue has stalled for many years.

CAARE will structure these comments to directly address the questions posed by Dr. Casey in his essay "In The Spotlight" on AltTox.org of August 25, 2016. ¹

1. In the absence of sufficient human data, how can new methods be validated as having equivalent (or better) performance than the animal-based test without a direct comparison to data from the animal test intended for replacement?

This question elicits the underlying conundrum with validating and implementing non-animal methods (hereafter NAMs), due to inevitable disparities when comparing outcomes of animal vs. non-animal tests.

Simply put, animal tests have generally failed to predict toxicity of chemicals and other xenobiotics, which is why there are currently an estimated 84,000 chemicals in use without known or conclusive human data. This gap exists despite six or seven decades of dedicated animal testing.

If human-based tests are more predictive – as they have been shown to be – it stands to reason that they will yield different results than animal tests. This makes it impossible to evaluate NAMs by direct comparison of the results arising from animal assays.

What is needed are methods that measure NAMs on their inherent scientific ability to predict human biologic outcomes without comparison to animal tests. Equally important, we need a regulatory system in support of that approach.

Evaluate NAMs on their inherent scientific abilities

While this point has stymied validation for many years, it need not. The issue of evaluating scientific quality on its own merits is not impossible. ICCVAM needs to undertake research into this area, by soliciting input from scientists in a broad range of areas.

Additionally, since global advances in this area have surpassed the U.S., one starting point might be to investigate validating methods used in other countries that have implemented many more NAMs. This would include those countries where animal testing bans are in place.

When evaluating NAMs without comparison to animal tests, here are some criteria to consider.

Evaluate NAMS based on their ability to yield known results for human response.

Evaluate assays on their ability to deliver results that are already well-known and documented. Scientists are doing this with organoids and organs-on-chips to provide proof-of-concept.

- Biotech company Organovo published a study to demonstrate that its 3D liver model can distinguish between the hepatotoxic compound Trovafloxacin, and the closely related non-toxic drug Levofloxacin. ²
- To evaluate their mini-brain model, researchers at Johns Hopkins tested the response of the model to Rotenone, a pesticide, and MPTP (methyl phenyl tetrahydropyrimidine), both of which are known to induce symptoms of Parkinson's disease in humans and animals. The mini-brain recreated the death of dopaminergic neurons characteristic of Parkinson's disease. ³

Reproducibility: A predictive test must be able to be repeated in any lab, at any time, under similar conditions, and give results that are identical within a reasonable scientific error of margin. (Animals test are notable for falling short of this criterion)

A reproducibility score can be developed based on a test's ability to perform in a designated number of labs, and that score used to evaluate its success or failure.

Most efficient methods should receive priority for validation

Data should be generated for assays on speed, cost and efficiency. These can be compared amongst tests. If NAMs are performing significantly better in these areas, they are guaranteed to generate superior results, just by their ability to assess greater numbers of chemicals at lower cost.

A new mindset is needed

Rather than struggling to match results from imprecise animal studies with advanced, innovative modern science, it is time to consider abandoning the comparison to animal tests entirely, and evaluate NAMs solely on their own ability to perform.

This needs a fresh mindset, which is an obstacle to government, in particular regulatory agencies, which tend to be rooted in convention.

2. What are some potential solutions to facilitate the use of human data in the future?

Availability of human tissue

Lack of available human tissue for research is one significant factor that causes investigators to rely on animal studies in place of human research. A recent study in the UK examined the value of human tissue for asthma research and demonstrated a need for fresh, whole human lung tissue with simultaneous barriers to obtaining this tissue.

As a result, a collaboration of institutions has been established with funding from the National Centre for the Replacement Refinement & Reduction of Animals in Research, "... to open up a pathway to obtain fully ethically consented, human (normal and diseased) lung tissue for the UK scientific community, thereby reducing the need for animal tissue in research. This collaboration will investigate lung tissue but could be expanded to include almost every tissue or organ type in the body." ⁴

Accordingly, NIH needs to establish a human tissue repository to facilitate the ready availability of ethically obtained specimens for human relevant research. Currently, the U.S. has only privately operated tissue banks. The exception is the NIH NeuroBioBank (NBB), established in September 2013, "to facilitate the distribution of high-quality, well-characterized human-post mortem brain tissue for the research community." ⁵ More tissue banks will increase work in this area.

Microdosing

Microdosing is a relatively new methodology that appears untapped in its potential for use in human toxicology and pharmacology studies. Employed in what are termed Phase 0 clinical trials, these first-in-human exposures utilize microdoses at typically “1% of the pharmacologic dose or 100 µg, whichever is lower, to human subjects to attain pre-phase1 pharmacokinetics (PK) in humans.”⁶

As described in a 2015 publication,

Over the past few years microdosing has found utility in pediatrics, protein-based therapeutics, and a new application known as intra-arterial microdosing that focuses more on localized pharmacodynamics than PK. Compared with other PK predictive methods, such as physiologically based pharmacokinetic modeling, allometry, and in vitro-in vivo extrapolation, microdosing appears to provide a significantly better understanding of PK prior to phase 1, albeit within what is currently a limited database.⁷

Analysis of these sub-pharmacological doses is carried out using sophisticated tools such as accelerator mass spectrometer (AMS), Positron Emission Tomography (PET), and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).⁸

While more typically used in pharmacology, microdosing has also been shown to have applications for toxicology. A 2015 publication described a study that used oral microdosing to determine the pharmacokinetics of Dibenzo(def,p)chrysene (DBC), a polycyclic aromatic hydrocarbon without prior known effect in humans.⁹

3. What strategies and mechanisms could be employed to increase communication and coordination of activities amongst and between the federal government and key stakeholders?

A New Memorandum of Understanding

In order to see necessary progress, it must be a priority for all parties involved. Presently, this does not appear to be the case. A noteworthy attempt to bring relevant organizations together to modernize toxicity testing was achieved, to some extent, by the 2008 Memorandum of Understanding between EPA, NTP and NIH. The FDA was added in 2010.

CAARE believes that a new Memorandum of Understanding is needed to re-prioritize many of the issues that were addressed in 2008, as well as incorporate and update new issues that have arisen, or have been thus far inadequately addressed.

The priorities of the 2008 MOU are worth re-stating:

The MOU provides for sample and information sharing necessary to more rapidly and effectively identify chemicals that might pose possible risks to the health of humans and animals and to the environment.

The MOU and the plans articulated in the *Science* article provide a framework to implement the long-range vision outlined in the 2007 National Research Council (NRC) report, *Toxicity Testing in the 21st Century: A Vision and a Strategy*, which calls for a collaborative effort across the toxicology community to rely less on animal studies and more on *in vitro* tests using human cells and cellular components to identify chemicals with toxic effects. Importantly, the strategy calls for improvements in dose-response research, which will help predict toxicity at exposures that humans may encounter.¹⁰

The 2008 MOU was stated to be in effect for five years, which means its term ended in 2013. This further underscores the need for a new agreement. ICCVAM should be included in the new agreement.

An updated MOU should include a timeline for achieving goals. It should also stipulate the creation of a steering committee to monitor progress, identify roadblocks and develop strategies to circumvent obstacles as they arise. If possible, the steering committee should allow for participation by animal welfare groups who are working on this issue.

This brings us to our next point: the creation of a new and separate agency having the sole function to develop alternative testing methods to replace animals.

There can be no question that the creation of a separate agency within the U.S. Department of Health and Human Service, dedicated to developing, implementing, and funding alternatives to animal tests is the best way to accomplish the goal of efficient replacement of outdated animal tests. Indeed such an agency is warranted and badly needed.

The agency would be comparable in operation to the UK's National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs).

Here is a segment from the NC3R website that describes its mission:

We collaborate with scientists and organisations from across the life sciences sector, nationally and internationally, including universities, the

pharmaceutical, chemical and consumer products industries, other research funders, and regulatory authorities.

We support the commitment of the scientific community to the 3Rs by funding research and early career development, supporting open innovation and the commercialisation of 3Rs technologies, and stimulating changes in policy, regulations and practice.

We are the main funder of 3Rs research in the UK with over £45 million committed since 2004. We have a range of schemes to support projects, infrastructure, and training and career development.

If the U.S. is to remain truly competitive on the world stage in developing cutting-edge non-animal methods that supersede outdated and ineffective animal models, then the creation of a separate agency is arguably the only way to accomplish this.

Barring that, and until which time a comparable U.S. agency is established, the following will aid in overcoming current obstacles to implementing modern, human-based science assays:

Impose requirements

Establish clear requirements to implement human-based assays in place of animal tests through policy, regulations and laws, which mandate the use of alternatives to animal tests.

The Frank R. Lautenberg Chemical Safety for the 21st Century Act, which was recently enacted, calls for increased use of information from alternative test methods and strategies. It is a good first step, but more legislation and clear requirements are needed.

Funding

Government should provide funding for non-animal methods

Funding should go towards devising improved human cellular models, and for developing sophisticated, interconnected organs-on-chips to better model the intact living system. Bioinformatics, in silico medicine, computer modeling and artificial intelligence also deserve more funding to explore their predictive capabilities.

The NIH should offer grants that pay for training to shift scientists to NAMs. This is needed to overcome the barrier of skilled scientists who lack expertise in non-animal methods.

Some of this is already happening, but more is needed.

Willingness to change

FDA must accept non-animal test results

Scientists and researchers can hardly be open to change when the agencies they must comply with are not. The FDA should end its animal testing mandate and accept proof-of-concept for NAMs where manufacturers and scientists can provide them.

4. What are the most important “non-scientific” issues and how should they be prioritized?

There are numerous issues outside of scientific effectiveness that affect progress in moving to modern, human-based science. In CAARE’s opinion, the most important of these is the role of government and regulations arising from policy. Government sets policy, and establishes the framework for what is accepted. In general, people look up to government as the authority, particularly in the area of science and medicine.

Similarly, political issues are important and impact on this area, as politicians and lawmakers work in tandem with government.

Another significant factor is academia, which is a huge driver of research, including toxicology. Academic researchers are under constant pressure to publish. The ready availability of animals, combined with the relative ease of generating animal tests, promotes the steady use of animals. Animal studies are generally easier to carry out, being less demanding and complicated to set up. In contrast, human studies involve informed consent, and the search, screening and recruitment of appropriate volunteers, along with more complex steady design that includes requirements for randomization and double-blinding. We *test* on animals, but we do human *research*.

One way to deal with this is to impose greater requirements and oversight for animal research. The question becomes, who will carry out that oversight, and even before that, laws are needed, or at the very least, policies that will require greater oversight and requirements. Here is where a dedicated agency to promote NAMS could act.

These are complex issues which point to the need for systemic and far-reaching change in a number of areas, which is why this has been so difficult to bring about.

Role of animal advocacy groups to effect change

Though change works from the top down, it also occurs from the bottom up. Laws get passed when the public demands change and prevails upon legislators to heed their concerns. Change is a multidirectional event, with forces coming from many routes, which is why it takes years. Greater inclusion of animal welfare organizations will facilitate change because their primary mission is generally advocacy. This is not the role of government.

The lengthy time involved should not allow us to become cynical or resigned. Like many other things, change happens exponentially, as cracks form in the foundations that prop up outdated systems. Once those cracks reach a critical level, walls can come down.

Concluding remarks

Progress has been made in the U.S. toward acknowledging the need to advance the use of human-based predictive tests. The present meeting and its focus on developing strategy and implementation toward that end are encouraging. Yet a great deal more work needs to be done to align the stated goals of the participating agencies with the reality of the state of science in 2016.

CAARE was disappointed to read the report of a large-scale, \$25 million study in rats carried out by NTP to determine cell phone safety. The study results were released in May 2016 with more results expected as analyses are completed. ¹¹

Rats were subjected to high level radio- frequency radiation of the type emitted from cell phones for nine hours a day, beginning in utero and continued over two years.

As with so many animal studies, the findings were of ambiguous significance. Tumors showed up in numbers that may or may not have statistical significance. Confounding the data even further, the results showed up only in male rats without explanation.

The New York Times article reported that the animal studies contrast with two extensive studies of human exposure, and studies on human cells that have showed no adverse effects. A number of other human studies have shown similar results. In response to the rat study, health officials are not modifying their current position on cell phone safety.

This study was initiated in 2009 and we can only hope that this is the last of its kind that the NTP plans to conduct. \$25 million would go a long way toward building a human tissue biobank or funding studies to establish the efficacy and safety of microdosing, among many other things.

CAARE appreciates the opportunity to submit these comments, and the interest and commitment of the participating agencies.

Sincerely,
[Signature Redacted]

Barbara Stagno, RN
President
Citizens for Alternatives to Animal Research & Experimentation

References

- ¹ Casey, Warren, *In the Spotlight: Implementing the Vision for Toxicity Testing in The 21st Century: An Opportunity for Action*, at <http://alttox.org/implementing-the-vision-for-toxicity-testing-in-the-21st-century-an-opportunity-for-action/>
- ² Nguyen, DG, et al , *Bioprinted 3D Primary Liver Tissues Allow Assessment of Organ-Level Response to Clinical Drug Induced Toxicity In Vitro*, PLOS, July 7, 2016, <http://dx.doi.org/10.1371/journal.pone.0158674>
- ³ AAAS 2016: *Newly developed mini brains could dramatically alter drug discovery*. Bioworld, Feb 2016. <http://www.bioworld.com/content/aaas-2016-newly-developed-mini-brains-could-dramatically-alter-drug-discovery-0>
- ⁴ Edwards J, Belvisi M, Dahlen SE, Holgate S, Holmes A. *Human tissue models for a human disease: what are the barriers?* [Thorax. 2015 Jul;70\(7\):695-7](http://dx.doi.org/10.1186/s12931-015-0271-7)
- ⁵ The NIH NeuroBioBank (NBB). <https://neurobiobank.nih.gov/>
- ⁶ Lappin, G, *The expanding utility of microdosing*, [Clin Pharmacol Drug Dev. 2015 Nov;4\(6\):401-6. doi: 10.1002/cpdd.235. http://www.ncbi.nlm.nih.gov/pubmed/27137711](http://dx.doi.org/10.1002/cpdd.235)
- ⁷ Ibid.
- ⁸ Burt, T, John CS, Ruckle, JL, Vuong, LT; *Phase-0/microdosing studies using PET, AMS, and LC-MS/MS: a range of study methodologies and conduct considerations. Accelerating development of novel pharmaceuticals through safe testing in humans - a practical guide. Expert Opin Drug Deliv. 2016 Sep 1:1-16.*
- ⁹ Madeen E, et al, *Human in Vivo Pharmacokinetics of [(14)C]Dibenzo[def,p]chrysene by Accelerator Mass Spectrometry Following Oral Microdosing. Chem Res Toxicol. 2015 Jan 20;28(1):126-34. doi: 10.1021/tx5003996. http://www.ncbi.nlm.nih.gov/pubmed/25418912*
- ¹⁰ NIH News Release; *NIH Collaborates with EPA to Improve the Safety Testing of Chemicals, New Strategy Aims to Reduce Reliance on Animal Testing*, February 14, 2008, <https://www.nih.gov/news-events/news-releases/nih-collaborates-epa-improve-safety-testing-chemicals>
- ¹¹ Pollack, A. "Questions and Answers on the New Study Linking Cellphones and Cancer in Rats," *The New York Times*, May 27, 2016. http://www.nytimes.com/2016/05/28/health/cancer-study-radiation-cellphones.html?_r=0