



December 1, 2021

Dr Mary Wolfe; Designated Federal Official for the BSC.  
Office for the BSC, Office of Policy, Review, and Outreach.  
Division of NTP,  
National Institute of Environmental Health Sciences,  
PO Box 12233,  
Research Triangle Park, NC, 27709.

RE: National Toxicology Program Board of Scientific Counselors

Dear Dr Wolfe,

On behalf of the Humane Society of the United States (HSUS), Humane Society Legislative Fund (HSLF), and our members and supporters, we appreciate the opportunity to provide comments on FR Doc No: 2021-23916 “National Toxicology Program Board of Scientific Counselors; Announcement of Meeting” and the upcoming meeting of the National Toxicology Program (NTP) Board of Scientific Counselors (BSC) on December 8, 2021.

We have reviewed the currently active projects across the four discrete research areas: Exposure-based Research Programs; Health Effects Innovation Programs; Responsive Research Programs and Strengthening Capabilities Programs<sup>1</sup> and believe there is much to celebrate within these areas. For example, we are heartened to see non-animal methodologies such as systems biology approaches, *in vitro* assays, extensive use of Tox21 data, QSAR models, microphysiological systems, organoids and the specific inclusion of the new approach methodologies (NAMs) within the Developmental Neurotoxicity Health Effects Innovation element of the Health Effects Innovation Program<sup>2</sup>.

However, we believe there are missed opportunities to use these human-relevant approaches more widely. Therefore, we appreciate the opportunity to offer feedback on these activities and where we feel the BSC can play a role; enhancing the research program through the wider adoption of more human relevant, predictive approaches that do not rely on animals.

---

<sup>1</sup> <https://www.niehs.nih.gov/research/atniehs/dntp/strategic-plan/index.cfm>

<sup>2</sup> <https://www.niehs.nih.gov/research/atniehs/dntp/strategic-plan/health/developmental/index.cfm>

To this end, we offer the following points of comment:

- We note that several of the existing projects include comprehensive literature reviews. These include scoping reviews of the effects of personal care products on pregnancy and fetal care<sup>3</sup>, systematic review of the possible cancer hazard assessments of nitro polycyclic aromatic hydrocarbons<sup>4</sup> and characterization of cardiovascular disease in under-represented populations<sup>5</sup>, to name but three. Scoping reviews like this are very useful tools for ensuring that research is cutting edge and does not duplicate existing efforts. We encourage NTP to carry out such comprehensive reviews prior to the initiation of any new projects.
- In addition to comprehensive literature reviews, retrospective analyses have proven useful for defining under which conditions animal data do not provide valuable information for human health risk assessments<sup>6,7,8</sup>. We strongly urge the BSC to request retrospective analyses of previous projects be performed for all projects that would require new animal testing before project approval. For example, a recent retrospective study comparing the capacity of rat *in vivo*, rat *in vitro* or human *in vitro* assays for predicting dermal absorption across human skin, the dermal absorption factor (DAF), revealed that the rat *in vitro* data were similar to *in vivo* and concluded that the “comparisons presented support potentially using *in vitro* data alone for DAF derivation for human health risk assessment of pesticides.”<sup>9</sup> Retrospective analyses could therefore save time and animals, and would inform a more effective and efficient project methodology.
- For any new project, we suggest that part of the internal project application and approval process incorporates a comprehensive analysis of areas where NAMs could be applied to reduce and potentially replace animal use. We suggest that the BSC is structured to provide expert input in this subject matter and offer guidance as to where to find these resources. This should include, but not be limited to, the National Institute of Health’s Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing (ALTBIB)<sup>10</sup>. Researchers could also consult NAT, the Non-animal technologies database<sup>11</sup>, the European Union Reference Laboratory European Centre for Validation of Alternative (EURL ECVAM) methods collection DB-

<sup>3</sup> <https://www.niehs.nih.gov/research/atniehs/dntp/strategic-plan/exposure/combined/index.cfm>

<sup>4</sup> <https://www.niehs.nih.gov/research/atniehs/dntp/strategic-plan/health/carcinogenicity/index.cfm>

<sup>5</sup> <https://www.niehs.nih.gov/research/atniehs/dntp/strategic-plan/health/cardiovascular/index.cfm>

<sup>6</sup> <https://www.epa.gov/sites/default/files/documents/immunotoxicity-retro-analysis.pdf>

<sup>7</sup> Strickland J., Paris M.W., Allen D., Casey W. (2019) Approaches to Reducing Animal Use for Acute Toxicity Testing: Retrospective Analyses of Pesticide Data. In: Kojima H. *et al.* (eds) Alternatives to Animal Testing. Springer, Singapore. [https://doi.org/10.1007/978-981-13-2447-5\\_5](https://doi.org/10.1007/978-981-13-2447-5_5)

<sup>8</sup> Linke, B., Mohr, S., Ramsingh, D. and Bhuller, Y. (2017) A retrospective analysis of the added value of 1-year dog studies in pesticide human health risk assessments. *Crit Rev Toxicol.* Aug;47(7):581-591. doi: 10.1080/10408444.2017.1290044.

<sup>9</sup> Allen, D.G., Rooney, J., Kleinstreuer, N., Lowit, A. and Perron, M. (2021) Retrospective analysis of dermal absorption triple pack data. *ALTEX.* 38(3):463-476. doi: 10.14573/altex.2101121.

<sup>10</sup> [https://ntp.niehs.nih.gov/whatwestudy/niceatm/altbib/index.html?utm\\_source=direct&utm\\_medium=prod&utm\\_campaign=ntpgolinks&utm\\_term=altbib](https://ntp.niehs.nih.gov/whatwestudy/niceatm/altbib/index.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=altbib)

<sup>11</sup> <https://www.nat-database.org>

ALM<sup>12</sup> and the RE-Place database<sup>13</sup>. In order to ensure that NAMs are fully incorporated into any new or existing projects, we suggest that membership of the BSC should include additional expert advice on where NAMs could be used.

- We note an impressive array of *in vitro* methods and other non-animal approaches are in use across the range of NTP projects; however, we are concerned that there may be missed opportunities to apply these approaches beyond a specific project. We envisage that the BSC meeting is an ideal occasion for sharing best practices across the portfolio of NTP projects, and we suggest that this offers an opportunity to further reduce animal use. When reviewing projects, BSC should consider where the innovative non-animal methods in one specific project or research area could be applied elsewhere. For example, we note that within the Carcinogenicity Health Effects Innovation there is a project developing colonic organoids as an *in vitro* model whereas, in the same program, other research areas are assessing genomic alterations in tumors using rats – an area where *in vitro* methods, specifically organoids, are being applied with some success<sup>14,15,16</sup>. The BSC review of the NTP programs, and specifically this meeting, could reveal cross-over opportunities to apply the non-animal methods more widely and we would encourage this.
- In order to fully exploit the advances that non-animal approaches are offering beyond the field of toxicology, we urge the BSC to use their wide expertise to offer suggestions where external collaborations may prove fruitful to accelerate the replacement of animal use across the NTP's program of research.
- We notice that one of the ongoing projects at NTP is investigating genetic diversity through genetic engineering of mice. We appreciate the need to understand the role that genetics and epigenetics play in the response to potential toxins and would respectfully suggest that it may be more powerful to try and exploit human cell banks, epidemiological studies, electronic health data or other sources of real-world data that focus on humans. We note that there is expertise in the BSC that may be able to offer suggestions on how to achieve this human-centric approach.
- We see the announcement of the EPA to eliminate mammalian testing by 2035 as the kind of progressive statement that embraces both public opinion to reduce animal use and the mounting scientific evidence of the failures of animals to predict human responses. We encourage the NTP to consider a similar ambitious statement and would urge the BSC to explore this and to work with the NTP to develop a roadmap, incorporating timelines for animal replacement across the NTP portfolio.

---

<sup>12</sup> <https://ec.europa.eu/jrc/en/scientific-tool/database-alternative-methods-animal-experimentation>

<sup>13</sup> <https://www.re-place.be/database>

<sup>14</sup> Lu, Z. Nie, B., Zhai, W. and Hu, Z. (2021) Delineating the longitudinal tumor evolution using organoid models J Genet Genomics. Jul 20;48(7):560-570. doi: 10.1016/j.jgg.2021.06.010.

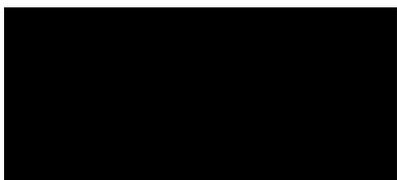
<sup>15</sup> Smith, R.C. and Tabar, V. (2019) Constructing and Deconstructing Cancers using Human Pluripotent Stem Cells and Organoids. Cell Stem Cell. Jan 3;24(1):12-24. doi: 10.1016/j.stem.2018.11.012.

<sup>16</sup> Yan, H.H.N. *et al.* (2020) Organoid cultures of early-onset colorectal cancers reveal distinct and rare genetic profiles. Gut. Dec;69(12):2165-2179. doi: 10.1136/gutjnl-2019-320019.

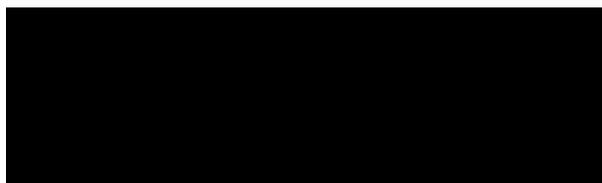
We appreciate the time and effort of the BSC in their review of the ongoing work and their input to the program of research. This meeting provides an opportunity to showcase the innovative, ground-breaking and animal-free methods that are currently in use at the NTP. However, the non-animal approaches should be embedded horizontally throughout all four research areas and not isolated in specific projects. The BSC, with their vision of the overall research areas, could use this meeting as an avenue for communicating where the non-animal approaches could be more widely used.

Thank you for the opportunity to provide comments. HSUS and HSLF appreciate the continued progress that the research at NTP is making and the input of the BSC to maximize the return on investment and ensure that their science is as relevant and human predictive as possible, by ensuring that the methods used across the entire suite of research projects are embracing the advances in non-animal science.

Sincerely,



Lindsay Marshall, PhD  
Biomedical Science Advisor  
Animal Research Issues  
The Humane Society of the United States



Gillian Lyons  
Director of Regulatory Affairs Federal Affairs  
Humane Society Legislative Fund