THE HUMANE SOCIETY OF THE UNITED STATES

HUMANE SOCIETY LEGISLATIVE FUND™

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Dr. Sheena Scruggs
Designated Federal Official for SACATM
Office of Policy, Review, and Outreach
Division of NTP, NIEHS
P.O. Box 12233, K2–03
Research Triangle Park, NC 27709

RE: Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments

Dear Dr. Scruggs,

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 in response to the June 30, 2021 notice "Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments" 86 FR 34771. The SACATM meeting agenda outlines two main topics for discussion, which we address individually here.

Ecotoxicology Testing

HSUS and HSLF were pleased to see that much of the meeting will be focused on the issue of ecotoxicity testing and are eager to learn more about the work of the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) work group on this subject. While great advances have been made in developing new approach methodologies (NAMs) that address human relevance through greater understanding of human biology, there is also a need to ensure that chemicals, both new and old, are safe for the environment. However, just as animal tests for human toxicity are time-consuming, variable, and cruel, so too are the traditional animal tests used to assess ecotoxicity.

During the 11th World Congress on Alternatives and Animal Use in the Life Sciences, a session about alternatives to fish toxicity testing provided insight on some of the modern approaches available to replace the traditional fish toxicity tests. During his presentation, Dr. Martin Paparella of the Medical University Innsbruck explained the main limitations of the acute fish toxicity test (OECD TG 203). In addition to conflicting with the principles of the 3Rs due to the

large number of fish used and lethality as the endpoint, the test has high variability, and does not provide mechanistic information or proven relevance to environmental conditions. By replacing this test with an Integrated Approach to Testing and Assessment (IATA), many of these limiting factors can be addressed. Paparella concluded that "to fully exploit the potential of alternative methods, the focus of regulatory toxicology needs to shift…towards development and harmonization of IATAs…built on highly standardized alternative methods supported by computational approaches."[1]

One such non-animal alternative method was described in a recently published OECD guideline, *Test No. 249: Fish Cell Line Acute Toxicity - RTgill-W1 cell line assay*. The test uses cell lines from the gills of rainbow trout to "(i) predict fish acute toxicity in product testing; (ii) range-finding and pre-screening before conducting a full fish acute or other fish-based toxicity test; (iii) generation of toxicity information to be used for hazard assessment in combination with other lines of evidences (e.g., Quantitative Structure Activity Relationships (QSAR), weight of evidence (WoE)) within Integrated Testing Strategy (ITS)/Integrated Approach to Testing and Assessment (IATA)."[2] However, as presented by Prof. Dr. Kristin Schirmer from the Swedish Federal Institute of Aquatic Science and Technology, rainbow trout cell lines may soon be used to replace not just the fish acute toxicity test, but also the fish early life stage test. In addition, the fish bioconcentration test could potentially be replaced by utilizing cell lines from the gills in combination with liver and intestine cell lines of rainbow trout.[3] Due to the large number of vertebrates used in the traditional fish tests and the increasing concern about environmental effects of chemicals, ICCVAM member agencies should be encouraged to prioritize the development and acceptance of NAMs-based IATAs (including adoption of methods employing fish cell lines) for ecotoxicology testing.

Another area of ecotoxicity testing that needs to be addressed is the use of multiple avian species for pesticide registration. Currently, for the avian oral toxicity test (TG 850.2100) EPA requires data from one passerine species and either one waterfowl species or one upland game bird species for terrestrial, aquatic, forestry, and residential outdoor pesticide uses. For the avian dietary toxicity test (TG 850.2200) and the avian reproduction test (TG 850.2300), data are required from a waterfowl species and an upland game bird species. While Environmental Protection Agency (EPA) Office of Pesticide Products (OPP) has recently issued guidance for

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[1] Paparella, M. (2021, August 24). *Limitations and uncertainties of acute fish toxicity assessments can be reduced using alternative methods* [PowerPoint presentation]. 11th World Congress on Alternatives and Animal use in the life sciences, Maastricht, Netherlands. https://www.wc11maastricht.org/

[2] OECD (2021), *Test No. 249: Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/c66d5190-en.

[3] Shirmer, K. (2021, August 24). *Fish cell lines of rainbow trout as alternatives to fish in environmental risk assessment: where we stand and where we need to go* [PowerPoint presentation]. 11th World Congress on Alternatives and Animal use in the life sciences, Maastricht, Netherlands. https://www.wc11maastricht.org/

waiving the dietary test,[4] and thereby reducing the number of birds used in pesticide registration, there is still the requirement for two species in the other two avian tests. Similar to the retrospective analysis done on utility of the avian dietary test, we encourage ICCVAM and EPA to review the value of a second species in these tests and investigate possible ways of using data from one species to predict outcomes in a second species.

Evolving Approaches to Validation

As ICCVAM and SACATM evaluate new approaches to test method validation, it is important for regulators to incorporate advances in science and toxicology and reconsider what information is most needed and relevant for human health risk assessment decisions. NAMs may not provide the same type of information that traditional animal studies provide; however, that does not mean NAMs do not address regulatory needs. In traditional animal tests, animals are used as surrogates for humans. When NAMs are developed based on an understanding of human biology, the data they provide will inevitably be more relevant to human safety concerns. HSUS and HSLF urge SACATM and all ICCVAM member agencies to consider the importance of developing and accepting integrated testing strategies, look critically at the relevance and usefulness of animal data, and utilize animal chip data to verify reliance of microphysiological systems (MPS).

Focus on testing strategies

As toxicity testing moves away from considering the concept of alternatives as one-for-one replacements of animal test methods, it is important to consider the many factors that may guide regulatory acceptance. As described by International Cooperation on Cosmetics Regulation (ICCR), a Next Generation Risk Assessment (NGRA) should adhere to nine principles for safety assessment without new animal data: 1. the overall goal is a human safety assessment; 2. exposure-led; 3. hypothesis-driven; 4. designed to prevent harm; 5. utilizes all existing information; 6. uses a tiered and iterative approach; 7. uses robust and relevant methods and strategies; 8. uncertainty should be characterized and documented; and 9. the logic of the approach should be transparent.[5] The NGRA model is an example of an IATA, which should be developed to satisfy the regulatory needs of ICCVAM member agencies. In its 2016 guidance document, the Organisation for Economic Co-operation and Development (OECD) explains the importance of IATAs being tied to adverse outcome pathways (AOPs) as it allows one to: "(a) evaluate in a structured way the existing information that is available for the chemical(s) of interest and possibly conclude on the hazard based on existing information; (b) identify and generate the type of information that might be required to increase the confidence

[4] Environmental Protection Agency (2020, February). Final Guidance for Waiving Sub-Acute Avian Dietary Tests for Pesticide Registration and Supporting Retrospective Analysis. Retrieved from: https://www.epa.gov/sites/production/files/2020-02/documents/final-waiver-guidance-avian-sub-acute-dietary.pdf

[5] Dent, Matthew et. al. (2018). Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients, Computational Toxicology. https://doi.org/10.1016/j.comtox.2018.06.001

level concerning evidence of a particular hazard; and (c) iteratively suggest which information is required to make a regulatory decision."[6] As agencies move forward with NAMs development, acceptance, and utilization, they should be integrating these new approaches into IATAs that incorporate a mechanistic understanding of the chemicals being evaluated. ICCVAM agencies should encourage their scientists to participate in AOP training, to better understand biological pathways and their role in determining risk. Such information is freely available from OECD.[7]

OECD explains that a defined approach (DA) "consists of a fixed data interpretation procedure applied to data generated with a defined set of information sources to derive a result that can either be used on its own, or together with other information sources within an IATA, to satisfy a specific regulatory need."[8] All ICCVAM member agencies should look at DAs as a way to clearly delineate a method for assessing chemical safety without the need for animal studies. For example, EPA's OPP and Office of Pollution Prevention and Toxics (OPPT) jointly released the 2018 *Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing*, which allows pesticide and industrial chemical manufacturers to choose one of two different DAs to determine skin sensitization without using animals.[9] This guidance offers the clarity that chemical and pesticide manufacturers need in order to utilize NAMs to assess skin sensitization.

Earlier this year, OECD also released Test Guideline 497, *Guideline for Defined Approaches on Skin Sensitisation*,[10] a first of its kind document from OECD that delineated DAs. The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) "led the project to develop the OECD guideline and worked with other U.S. government agencies as well as scientists from Canada and the European Union to sponsor the guideline."[11] Under the mutual acceptance of data, all OECD member countries must now

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[6] OECD (2016). *Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA*), OECD Series on Testing and Assessment, No. 260, OECD Publishing, Paris, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2016)67&doclanguage=en

[7] OECD (n.d.). *Adverse Outcome Pathways, Molecular Screening and Toxicogenomics.* Retrieved from: https://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm

[8] OECD (2017). *Guidance Document on the Reporting of Defined Approaches to be Used Within Integrated Approaches to Testing and Assessment*, OECD Series on Testing and Assessment, No. 255, OECD Publishing, Paris, https://doi.org/10.1787/9789264274822-en.

[9] US EPA Office of Chemical Safety and Pollution Prevention. (2018, April 4). Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing. Retrieved from: https://www.epa.gov/newsreleases/epa-releases-draft-policy-reduce-animal-testing-skin-sensitization

[10] OECD (2021). *Guideline No. 497: Defined Approaches on Skin Sensitisation*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/b92879a4-en.

[11] Sprankle, Catherine (2021, August). *New chemical testing approach will help to replace animal use*. Environmental Factor, National Institute of Environmental Health Sciences. Retrieved from: https://factor.niehs.nih.gov/2021/8/science-highlights/nonanimal-testing/index.htm

accept data generated from this DA. It is important that all ICCVAM member agencies quickly act to update their guidance to reflect their acceptance of data from the DAs outlined in this document for identifying skin sensitizers. The use of these types of testing strategies will continue to allow for the replacement of animal test methods and should reflect the future of validation.

Critical evaluation of animal data

When NAMS are scrutinized it is essential that the poor reliability of animal model data is acknowledged. NICEATM has been comparing results from animal data and non-animal testing strategies such as those published on skin sensitization[12] and acute oral toxicity[13] to build confidence in NAMs. A recognition of the inherent problems with variability and uncertainty in animal data needs to be considered when evaluating NAMs against this standard. Careful consideration of the actual value of animal data will enable federal agencies to minimize animal use without compromising human safety. It is important to remember that traditional animal tests have never gone through the same critical evaluation as the NAMs currently being considered.

We also ask NICEATM and ICCVAM agencies to regularly conduct retrospective analyses of data obtained for regulatory purposes to investigate whether the data were used in conducting hazard and risk assessments. In those instances where specific types of animal data were never or rarely used by the agency in regulatory decision-making, agencies should be encouraged to remove the requirement or publicize acceptance of waivers as EPA has done with the release in February 2020 of its *Final Guidance for Waiving Sub-Acute Avian Dietary Tests for Pesticide Registration and Supporting Retrospective Analysis*.[14] Critical review of the circumstances under which animal data were of value will enable regulators to make decisions based on weight of evidence without compromising human or environmental safety. It will also prevent agencies from wasting time developing NAMs to replace tests that do not provide additional value in risk assessment.

Utilizing animal chips to build confidence in MPS

There has been much interest by industry and regulators across the globe in the use of MPS to assess toxicity, test drug efficacy, and study disease. These promising technologies include the use of the Airway Chip to ascertain the best therapeutics to treat COVID-19 infection. The

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[12] Kleinstreuer, Nicole et.al (2018). Non-animal methods to predict skin sensitization (II): an assessment of defined approaches, Critical Reviews in Toxicology, DOI: 10.1080/10408444.2018.1429386

[13] Kleinstreuer, Nicole et.al (2018). Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation, Computational Toxicology, DOI: 10.1016/j.comtox.2018.08.002

[14] Environmental Protection Agency (2020, February). Final Guidance for Waiving Sub-Acute Avian Dietary Tests for Pesticide Registration and Supporting Retrospective Analysis. Retrieved from: https://www.epa.gov/sites/production/files/2020-02/documents/final-waiver-guidance-avian-sub-acute-dietary.pdf

airway chips "included cells that line the lung airway, blood vessel cells, and even immune system cells. The tissue that grew on the chips mimicked traits seen in living lungs, such as mucus production and inflammation."[15] While HSUS and HSLF believe that reliance on human biology-based NAMs such as the Airway Chip will provide better information than animal models, we encourage agencies to also invest in chip technology employing animal cells to assess the predictive capacity of these systems for chemical safety assessment. By directly comparing the results of animal chip data with animal data, regulators and industry can demonstrate the reliability of these MPS systems, allowing vital confidence building in human-chip data and speeding the phase-out of animal use. Animal chips could also be used to replace animal studies for veterinary drugs or to test the environmental impact of pesticides on non-target species.

Conclusion

As we have commented in past years, HSUS and HSLF continue to encourage significant, dedicated funding for NAMs development at all ICCVAM member agencies, international cooperation to ensure NAMs are accepted around the globe, and the proactive commitment with clear timelines to end reliance on animal test methods from all agencies. We welcome the opportunity to work with NICEATM or any ICCVAM agency to replace the use of animals with scientifically sound testing strategies. Thank you for the consideration of our comments.

Sincerely,

[redacted]

Vicki Katrinak
Director, Animal Research & Testing
Animal Research Issues
Humane Society of the United States

[redacted]

Gillian Lyons
Senior Regulatory Specialist
Humane Society Legislative Fund

[15] Reynolds, Sharon. (20201, May 25). *Airway-on-a-chip screens drugs for use against COVID-19.* NIH Research Matters. Retrieved from: https://www.nih.gov/news-events/nih-research-matters/airway-chip-screens-drugs-use-against-covid-19