

Corners

CAATfeed

Brussels Declaration on Ethics & Principles for Science & Society Policy-making

CAAT Director Thomas Hartung co-organized and contributed to the Brussels Declaration on Ethics & Principles for Science and Society Policy-making, a bottom-up approach on how scientific evidence is used to inform policy on issues related to science and society. The document brings together the findings from a series of five consultation events and symposia at global conferences from 2012-2016, in which more than 300 individuals from 35 countries examined how power operates in science and society.

This Declaration proposes a twenty-point blueprint for a set of ethics and principles to inform work at the boundary between science, society and policy. Its sole purpose is to boost understanding of how power operates and to explain why evidence plus dialogue rarely equals (as one might expect) good decisions and laws. Above all, it makes the case for a broad, multi-stakeholder and multi-disciplinary approach promoting greater integrity and accountability. The main recommendation for promoting public dialogue and better understanding is not only greater transparency and scrutiny, but genuine inclusivity.

Read the full Declaration at http://bit. ly/2nr3vTg.

Thomas Hartung Receives the Björn Ekwall Memorial Award

CAAT Director Thomas Hartung has received the Björn Ekwall Memorial Award for the year 2017 in recognition of his scientific achievements in the development and

evaluation of methods for risk assessment of toxic chemicals, without the use of experimental animals, and for his work as the Director of ECVAM (2002-2008) and the Director of CAAT (from 2009 until present). The Scandinavian Society for Cell Toxicology (SSCT) established the Björn Ekwall Memorial Foundation in 2001. The main goal of the BEMF is to honor the memory of Dr Björn Ekwall by giving a reward to scientists who have substantially contributed to the field of cell toxicology, e.g., by developing new *in vitro* tests, or via mechanistic or validation studies.

Marcel Leist Receives the GT-Toxicology Award

CAAT-Europe Director Marcel Leist has received the GT-Toxicology award for the year 2017 in recognition of his scientific publications in the field of *in vitro* toxicology, in particular for developing test methods for neural crest cells and for the peripheral nervous system. The award is sponsored jointly by the journal *Toxicology* and by the German Society of Toxicology. The award was presented during a festive ceremony at the German Toxicology summit in Heidelberg (Germany) on March 8, 2017.

Call for Pre-proposals: 2018-19 CAAT Research Grants

Deadline: April 21, 2017

CAAT's research grants program is a centerpiece of our work, providing initial funding for scientists to develop alternatives to the use of animals in biomedical research and product safety testing. To date, the center has funded some 300 grants (including renewals) for a total of more than \$6 million.

Although relatively small individually, these grants offer critical seed money that allows researchers to demonstrate the value of a particular area of study so they can gain support from the NIH and other sources.

Details: http://caat.jhsph.edu/programs/grants/index.html

EBTC has been Awarded a \$50,000 Prize from the Beagle Freedom Project

On February 2, the Beagle Freedom Project announced the Evidence-based Toxicology Collaboration (EBTC), for which CAAT provides the secretariat, as one of the winners of their first Funding the Future competition. The new EBTC project will use, for the first time, evidence-based methodologies to compare drug-induced liver toxicity in human patients to that of other experimental animals, including beagles, and to US EPA ToxCast in vitro data. A new EBTC workgroup that will include stakeholders from academic, industry, and non-profit sectors, will perform a systematic review of the literature ("study of studies") on ten marketed drugs that have reported adverse liver events, or lack thereof, in humans. This study aims to increase our understanding of interspecies differences frequently observed in adverse drug reactions and to explore the predictive ability of the EPA Tox-Cast program's in vitro tests for prediction of such human adverse events. The results will provide a direct objective comparison of the effects of these medicines on different species and may provide evidence inform-



ing regulatory agencies' decisions on tests that truly protect human health. Currently, regulatory agencies worldwide require the use of beagles and other animals to ensure human safety. Many studies have found that tests on animals fail to predict how drugs affect humans.

EBTC's work is funded by organizations committed to funding unbiased academic research regardless of the outcome. Like all of EBTC's funding, this award is unrestricted, and the funding organization will have no influence on the outcomes of the study and will take no part in research, data analysis, or interpretation.

US Travel Issues for CAAT Personnel

To our regret, a German CAAT team member of Iranian descent recently fell victim to changes in US travel policies and was unable to attend the annual Society of Toxicology meeting in Baltimore. Our colleagues and friends in the scientific community may want to be extra judicious in planning their travel to the US in light of this unfortunate situation. We ask that you make us aware if your travel has been complicated or made impossible due to changes in US law, as Johns Hopkins University is closely monitoring these instances. For attendees of the upcoming 10th World Congress on Alternatives and Animal Use in the Life Sciences, timely application for visa/waiver is strongly encouraged.

Martin Stephens et al. Article on Systematic Review Among Top 10% Most Downloaded Articles in Toxicological Sciences

View the article, "Emergence of Systematic Review in Toxicology," here: https://doi.org/10.1093/toxsci/kfw059.

Georgina Harris Receives Special Merit Award from the International Foundation for Ethical Research (IFER)

CAAT's Georgina Harris was awarded a fourth-year Graduate Student Fellowship

as a Special Merit Award for her project "Identification of pathways of developmental neurotoxicity (DNT) of environmental chemicals by omics technologies." The \$15,000 grant was awarded by the Scientific Advisory Committee, the Board of Directors, and the supporters of The International Foundation for Ethical Research (IFER). Please join us in congratulating Georgina!

Upcoming Meetings

4th Symposium on Social Housing of Laboratory Animals

May 1-2, 2017 Atlanta, GA

2nd Workshop on Macaque Pair Housing

May 3-5, 2017 Atlanta, GA

The symposium and workshop are co-hosted by USDA-AWIC, OLAW, The Centers for Disease Control, Yerkes National Primate Research Center, CAAT, and the Johns Hopkins School of Medicine Department of Molecular and Comparative Pathobiology.

The conference begins with two days of didactic content, interactive discussion, and problem-solving regarding methods of social housing for common laboratory species. Symposium day two concentrates on nonhuman primates and starts the 3.5 day interactive workshop focusing on the same. Participants may register for the 2-day symposium, the nonhuman primate workshop, or both.

Details and Registration: http://caat. jhsph.edu/programs/workshops/social_housing.html

CAAT Academy Announces Upcoming Hands-On Training in Toxicology Sessions

CAAT Academy is back in 2017 with a new team and more than eleven hands-on training sessions in toxicology across Europe beginning in June.

Full list of sessions: https://www.caat-academy.org

10th World Congress on Alternatives and Animal Use in the Life Sciences

August 20-24, 2017 Seattle, Washington

Details: http://wc10 seattle.org/2017/home.

aspx

Recent Meetings

CAAT-Europe Talk at Italian Parliament (Rome) on Regulatory Acceptance of *In Vitro* Approaches

March 15, 2017 Rome

The association OSA (Oltre la Sperimentazione Animale, Beyond animal experiments) sponsored an important event at the Italian Parliament in Rome (Italy) to present new opportunities for alternatives to animal experiments in the scope of biomedical research ("Ricerca biomedica: l'avanguardia dei metodi sostitutivi alla sperimentazione animale"). Invited speakers discussed the limits of the traditional *in vivo* tests and the answers that may arrive from advanced technologies combining computational science and in vitro 3D models. Costanza Rovida from CAAT Europe talked about the way forward to full acceptance by regulators of the latest in vitro approaches.

CAAT Events at Society of Toxicology Annual Meeting

UL First Annual Pub Night at ToxExpo

Sunday, March 12, 2017 Baltimore

SOT Satellite Meeting: Updates on Activities Related to 21st Century Toxicology and Related Efforts: Invited Presentations and Open Microphone

March 16, 2017 Baltimore

Regulatory Acceptance of Read-Across

March 17, 2017 Baltimore

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Recent Publications

Bowman, C. E., Zhao, L., Hartung, T. and Wolfgang, M. J. (2016). Requirement for the mitochondrial pyruvate carrier in mammalian development revealed by a hypomorphic allelic series. *Mol Cell Biol 36*, 2089-2104. https://doi.org/10.1128/MCB.00166-16

Escher, B. I., Hackermüller, J., Polte, T. et al. (2017). From the exposome to mechanistic understanding of chemical-induced adverse effects. *Environ Int 99*, 97-106. https://doi.org/10.1016/j. envint.2016.11.029

Hartung, T. (2017). Utility of the adverse outcome pathway concept in drug development. *Expert Opin In Drug Metab Toxicol 13*, 1-3. https://doi.org/10.1080/17425255.2017.1246535

Hartung, T. (2017). Evolution of toxicological science: The need for change. *International Journal of Risk Assessment and Management* 20, 21-45.

Juberg, D. R., Knudsen, T. B., Sander, M. et al. (2016). FutureTox III: Bridges for translation. *Toxicol Sci 155*, 22-31 https://doi.org/10.1093/toxsci/kfw194.

Maertens, A., Bouhifd, M., Zhao, L. et al. (2017). Metabolomic network analysis of estrogen-stimulated MCF-7 cells: A comparison of over-representation analysis, quantitative enrichment analysis and pathway analysis versus metabolite network analysis. *Arch Toxicol* 91, 217-230. https://doi.org/10.1007/s00204-016-1695-x

Myint, L., Kleensang, A., Zhao, L. et al. (2017). Joint bounding of peaks across samples improves differential analysis in mass spectrometry-based metabolomics. *Anal Chem 89*, 3517-3523. https://doi.org/10.1021/acs.analchem.6b04719.

Pamies, D. and Hartung, T. (2017). 21st century cell culture for 21st century toxicology. *Chem Res Toxicol* 30, 43-52. https://doi.org/10.1021/acs.chemrestox.6b00269

Pendse, S. N., Maertens, A., Rosenberg, M. et al. (2017). Information-dependent enrichment analysis reveals time-dependent transcriptional regulation of the estrogen pathway of toxicity. *Arch Toxicol 91*, 1749-1762. https://doi.org/10.1007/s00204-016-1824-6

Schmidt, B. Z., Lehmann, M., Gutbier, S. et al. (2016). In vitro neurotoxicity screening: An overview of cellular platforms and high-throughput technical possibilities. *Arch Toxicol 91*, 1-33. https://doi.org/10.1007/s00204-016-1805-9.

Tong, Z.-B., Hogberg, H., Kuo, D. et al. (2017). Characterization of three human cell line models for high-throughput neuronal cytotoxicity screening. J *Appl Toxicol 37*, 167-180. https://doi.org/10.1002/jat.3334

Zander, N. E., Piehler, T., Hogberg, H. and Pamies, D. (2017). Explosive blast loading on human 3D aggregate minibrains. *Cell Mol Neurobiol*, Epub ahead of print. https://doi.org/10.1007/s10571-017-0463-7



ecopa Annual General Assembly

ecopa and the Scandinavian Society for Cell Toxicology will hold a joint workshop in Helsinki (Finland) on June 14-16, 2017. The topic will be "Up-to-date in vitro approaches in regulatory risk assessment and disease modeling". More information is available on http://www.ficam.fi. The 18th General Assembly of ecopa will be held on June 14 at the same location from 17:00 to 19:00 and will elect a new board.

ecopa featured in the next ECHA newsletter

Our *ecopa* contact point at the ECHA stakeholder forum, Costanza Rovida, will be interviewed and will provide insights to ECHA readers about *ecopa* and the 3Rs as well as on how to employ 3Rs for registration purposes.

ecopa registered as EFSA Stakeholder

On February 24, 2017, *ecopa* received an official letter from the head of the external relations unit announcing the acceptance of *ecopa* as an EFSA (European Food Safety Authority) Stakeholder. The activities of EFSA embrace the assessment of any risk related to the food chain, including evaluation of all food and feed additives, plant protection products and welfare of farm animals.

The four concerned parties in the field of alternative methods to animal experiments, i.e., academia, animal welfare, industry and government are combined to compose the national consensus platforms represented in ecopa. Each of these platforms may have a strong interest in the food and feed sectors even though the main focus remains on the methodology for risk assessment rather than the evaluation of the single product. Within EFSA, ecopa now has the possibility to recommend the use of alternative strategies, in particular to elucidate the mechanisms of action of the different substances in humans. Animal models may fail to predict an effect in humans, especially in the area of nutrition where so many differences exist between humans and animals.



The possibility to enter into the decision mechanism of EFSA is a unique opportunity to increase visibility of alternative methods and improve their implementation.

ecopa welcomes ROCAM as a new associate member

ROCAM, the Romanian Center for Alternative Methods, joined *ecopa* in March 2017. ROCAM was established in June

2015 and is hosted by the University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca at the Institute of Life Sciences. More information about ROCAM is available at: http://rocam.usamvcluj.ro/.

FRANCOPA

 The French platform FRANCOPA has recently published the updated version of its report on the state of the art of 3Rs

- approaches in France. The report (in French) is available on the platform's website: http://www.francopa.fr/
- Professor Jean-Claude Nouët has retired from the management committee of FRANCOPA and Henri-Michel Baudet now represents the ONG LFDA.
- The annual meeting of FRANCOPA will take place on June 20 in Paris. It will be focused on organoids and ex vivo approaches.

[::::] EUTOXRISK

After the first project year, there was eager anticipation to hear the echo of the field during the international meeting of the Society of Toxicology (SOT). EU-ToxRisk was present with an information booth during the 56th annual SOT meeting from March 12 to 16 in Baltimore, USA. The interest in the project and its updates was overwhelming. Besides the booth, EU-ToxRisk science was communicated in 9 presentations, 16 posters and dedicated workshops.

The SOT meeting provided an appropriate platform for introduction of the project to interested colleagues from the US, including representatives of Tox21 and the US FDA. Communication channels to the Tox21 consortium were established already in September 2016 at a face-to-face meeting in Germany. During the SOT, a follow-up meeting with representatives of the Tox21 consortium was organized to further elaborate collaboration.

Currently, the EU-ToxRisk consortium is working on 8 case studies. These have been described in the previous

EU-ToxRisk corner in ALTEX and in the newsletter of the project (http://bit. ly/2nmW7t2). Some updates on the technical progress of the studies shall be provided here:

Case study 1 (CS 1) focuses on microvesicular liver steatosis using valproic acid (VPA) as a lead compound; test methods including PBPK modelling for prediction of plasma concentrations are already established. An inter-laboratory study has been conducted and could confirm the comparability of results. *In vitro* data generation involving HTS, cell-based, *ex vivo*, and organ-on-chip approaches are ongoing. The focus is on providing an initial read-across document and on identifying knowledge gaps and uncertainties to be addressed by EU-ToxRisk *in vitro* data.

Case study 2 (CS 2) focuses on teratogenicity of VPA and other branched carboxylic acids; the initial risk assessment based on *in vivo* data will be compared to *in vitro* testing data on the VPA analogues in test battery models. This will be followed by PBPK modelling. The battery approach and

PBPK findings will clarify if the approach predicts the *in vivo* response correctly.

CS 3 aims at the oxidative stress and liver toxicity of phenols/hydroquinones and resorcinols; PBPK modelling and *in vitro* models were used to establish the concentration range for omics approaches. The studies on combination of chemical and biological descriptors for read-across purposes as well as *in vitro* ADME data generation are ongoing.

CS 4 is concerned with mitochondrial toxicity; all test methods are in place and in use for mitotoxic pesticides, insecticides and fungicides.

CS 5 evaluates peroxisome proliferation and organic anion transporter interference (due to phenoxy carboxylic and acetic/propionic acids); the experimental work is about to start.

CS 6 has a focus on the prediction of blood concentrations of chemicals that cause an increased risk of hepatotoxicity; a set of 12 hepatotoxic and 9 non-hepatotoxic drugs were employed and in a second testing period 12 additional positive

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and negative controls are being tested. The study aims to refine the set of biomarkers and readouts for hepatotoxicity prediction.

CS 7 deals with read-across evaluation of reproductive toxicity of conazoles; the experimental work has been started with assays focusing on endocrine disruption, and the first data are already available.

CS 8 is concerned with the "popcorn lung", i.e., respiratory effects of diketones; for this case study the compound selection is completed and agreed on as well as the methods and the testing protocols. The AOP modeling process for all case studies is progressing continuously in parallel with data generation and integration of the information.

Besides the case studies, a large cross system study is running. It tests 19 compounds across 12 different cell models. This will provide a basis for quality assurance procedures for test methods as well as data handling and storage. Moreover, it will provide an important basis for repeated dose testing strategies and the EUToxRisk transcriptomics platform, apart

from showing the range of models available in the consortium.

Based on the scientific progress, the consortium carries on with a spirit of optimism. The achievements so far fully agree with the schedule of the project and all milestones have been reached in good time. Further progress will be shared in the next issue and on other platforms (at twitter (@EU_ToxRisk) and Facebook (public group and page).

Mardas Daneshian





ICCVAM Public Forum to Discuss New Approaches for Evaluating Chemical Safety

ICCVAM is coordinating an effort to explore new approaches for evaluating the safety of chemicals and medical products in the United States. The vision is that this effort will increase confidence in alternative methods and improve their relevance to human health outcomes while maximizing efficiency and maintaining a commitment to replace, reduce, and refine animal use. This collaborative effort by U.S. Federal agencies, the regulated community, non-governmental organizations and other technical experts will (1) help guide the development of new tools, based on new science and technology, to support regulatory and research needs, (2) use knowledge of human and animal biology as appropriate for helping to establish confidence in new approaches, and (3) help facilitate and encourage the implementation and use of these new approaches by Federal agencies and regulated industries.

This topic will be the major focus of the May 23 ICCVAM Public Forum, and interested stakeholders are invited to attend and submit comments. The meeting will be at the National Institutes of Health in Bethesda, MD, and will also be webcast. Meeting details and a link to registration will be available in April at http://ntp.niehs.nih.gov/go/iccvamforum-2017.

New Agency Joins ICCVAM

The National Institute of Standards and Technology (NIST) has joined ICCVAM. NIST participation will provide ICCVAM with interest and experience in the study of process controls, measurement artifacts, and interlaboratory testing.

ICCVAM was established to facilitate and promote development and regulatory acceptance of new toxicological tests with the potential to replace, reduce, or refine animal use. The expertise within NIST will benefit ICCVAM, especially in the development of validation studies to assess the appropriateness of new test methods for specific purposes. In particular, NI-ST has experience with cell-based and small model organism assays, which are becoming increasingly important as alternatives for traditional animal tests. NIST also brings to ICCVAM additional expertise in experimental design and statistical analysis.

This event represents the first time since its inception in 2000 that ICCVAM has expanded its membership to include a new



member agency. NIST, which is part of the U.S. Department of Commerce, has been interacting with ICCVAM since 2015. The agency submitted an official request to join ICCVAM in January 2017, which was approved in February by National Institute of Environmental Health Sciences Director Linda Birnbaum.

More information about NIST is available on its website at https://www.nist.gov/.

NICEATM Releases New Resource to Support Alternative Methods Development

NICEATM has launched the Integrated Chemical Environment (ICE), an online source of high-quality curated data and appropriate tools to support development and evaluation of new, revised, and alternative methods.

The ICE Data Integrator is a query tool that integrates curated *in vivo* test data, reference chemical information, *in vitro* assay data, and *in silico* predictions to facilitate hypothesis generation and testing. Data for about 10,000 chemicals are available; endpoints include acute oral toxicity, skin and eye irritation, skin sensitization, and endocrine activity. ICE also includes predicted physicochemical property data for about 40,000 chemicals. All data in ICE were generated using validated, nonproprietary test methods, are from high-quality experiments, and are publicly available with no restrictions on use.

Open-source downloadable computational software and workflows, which will become available in an update of ICE this summer, will enable *in silico* predictions to be made using either data obtained from the ICE Data Integrator or provided by the user. ICE is available at https://ice.ntp.niehs.nih.gov.

EPA Launches Voluntary Pilot Program to Reduce Animal Testing

In December 2016, the U.S. Environmental Protection Agency launched a voluntary pilot program to evaluate the usefulness and acceptability of the GHS Mixtures Equa-

tion, and published guidance on how pesticide companies can submit data for the program. This program is another step toward EPA's goal of reducing animal testing by adopting better testing methods, as described in the March 2016 Letter to Stakeholders issued by Office of Pesticide Programs Director Jack Housenger.

The GHS Mixtures Equation, a mathematical tool that estimates the toxicological classification of a chemical, is used in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). Use of the GHS Mixtures Equation can reduce animal use for oral and inhalation toxicity studies of pesticide formulations.

EPA requests submission of acute oral and acute inhalation toxicity study data paired with mathematical calculations (GHS Mixtures Equation data) to support the evaluation of pesticide product formulations. EPA expects that the pilot program will run through mid-2017, but data analysis will begin sooner if enough data is received.

More information about the pilot program and instructions for submitting data are available on the EPA website at https://www.epa.gov/pesticide-registration/mixtures-equation-pilot-program-reduce-animal-testing.

National Academies Report: Using 21st Century Science to Improve Risk-related Evaluations

A report released January 5 by the National Academies of Sciences, Engineering, and Medicine makes recommendations on the best ways to incorporate emerging science into risk-based evaluations of chemical safety. The report, "Using 21st Century Science to Improve Risk-related Evaluations", was prepared at the request of the four Tox21 partner organizations: EPA, National Institute of Environmental Health Sciences, National Center for Advancing Translational Sciences, and the U.S. Food and Drug Administration. The new report discusses both the inherent opportunities and the challenges that will need to be met to achieve the vision described in two earlier National Research Council reports, "Toxicity Testing

in the 21st Century" and "Exposure Science in the 21st Century."

The new report can be read online or downloaded as a free PDF at https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations. National Academies Press also has a paperback edition of the report available for purchase.

NICEATM and ICCVAM Activities at 2017 SOT Annual Meeting

NICEATM and ICCVAM presented two special sessions at the 2017 SOT annual meeting in Baltimore, MD.

- "Developing an Implementation Strategy for Toxicity Testing in the 21st Century" provided an update on ongoing efforts towards developing a strategy for the safe, effective, and timely implementation of 21st century toxicity testing approaches in the U.S. This session began the public phase of the effort described above to explore new approaches for evaluating the safety of chemicals.
- "ICCVAM Tools for Validation and Regulatory Application of Alternative Methods" featured speakers from NICEATM, the National Library of Medicine, and the U.S. Environmental Protection Agency presenting overviews of their online resources available to support alternative methods development. The NICEATM presentation featured an overview of the new ICE resource described above.

In other SOT activities, ICCVAM representatives served as co-chairs for five platform sessions, NICEATM scientists were co-authors on 13 poster or platform presentations, and ICCVAM members were co-authors on 18 presentations. A full list of NICEATM and ICCVAM activities at SOT is available at http://ntp.niehs.nih.gov/go/niceatm-sot17.

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IIVS Presents Posters at SOT 2017

IIVS presented the following posters at SOT 2017, March 12-16 in Baltimore:

- Addressing the Assignment of US EPA Hazard Categories of Dermal Safety by a Revised Prediction Model of the Validated In Vitro Skin Irritation Test (OECD TG 439)
- Precision Digital Dispensing of Patterned Picoliter Quantities of Test Material onto Apical Surfaces of Human 3D-Reconstructed Airway Tissues Correlation of Two In Vitro EpiOcular Test Methods and Consumer Eye Irritation Data for Cleaning Products
- Using In Vitro Assays, the Direct Peptide Reactivity Assay (DPRA), Keratinosens (KS), and Human Cell Line Activation Test (h-CLAT) to Assess Skin Sensitization Potential of Electronic Cigarette Liquids

View these posters on the IIVS website at http://www.iivs.org.

IIVS to Expand Laboratory Space

This Spring IIVS will open its new respiratory toxicology laboratory. The new lab will allow exposure of *in vitro* models to particulates, aerosols, smoke, or gases that may be found in our environment or in new materials such as electronic nicotine delivery systems.

The state-of-the art exposure laboratory will provide ISO conditions through precise environmental controls. The new laboratory will expand our current output capacity while also providing IIVS with new inhalation-like exposure capabilities to augment our portfolio of non-animal methods.

IIVS Presentation at NAS

IIVS Principal Scientist for Respiratory Toxicology, Dr Holger Behrsing, was invited to present at the National Academies of Sciences, Engineering, and Medicine's (NAS) workshop on electronic nicotine delivery systems (ENDS), e-cigarettes. The workshop was held by a recently formed NAS committee convened by the FDA's Center for Tobacco Products. The committee has been asked to evaluate the available evidence of the health effects related to the use of ENDS and to identify future federally funded research needs. Dr Behrsing presented on the pragmatic use of 3D lung models to evaluate exposure-induced adverse effects that may contribute to pulmonary disease.

Dr Behrsing's presentation can be viewed at http://www.iivs.org.

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