Winter 2020 Webinar: How to Get to Safer Using Non-Animal Testing Methods

Wednesday, January 29, 2020 9:00 AM - 10:00 AM PT 12:00 PM - 1:00 PM ET

TOPICS IN ALTERNATIVES ASSESSMENT

Free Webinar Series Hosted by the Association for the Advancement of Alternatives Assessment

WELCOME!

Today's A4 webinar:

How to get to safer using non-animal testing methods

Goal - to better understand:

- what are new approach methods (NAMs) – why they are of interest and how are they being used
- considerations for using NAMs in the context of conducting alternatives assessments, and
- where we can learn more about NAMs, if interested



Today's facilitator Dr. Margaret Whittaker TEXSERVICES

Co-Chair, A4 Program Committee



Today's Speakers and Respondents



Pamela Spencer – VP Regulatory, Product Stewardship and Quality





Amy Clippinger – *Director*

PETA INTERNATIONAL

Advancing 21st Century Toxicology



Lauren Heine – Director of Safer Materials & Data Integrity





Shari Franjevic – GreenScreen[®] Program Manager





Webinar Logistics

- Due to the number of participants on the webinar, all lines will be muted
- If you wish to ask a question, please type your question in the Q&A box located in the drop down control panel at the top of the screen
- Questions will be answered at the end of the panel discussion
- The webinar is being recorded and will be posted with the slide deck on the A4 website: <u>www.saferalternatives.org</u>
- At the end of the webinar, we will launch a short evaluation survey to help us with future webinars



Introduction to NAMs and Application of NAMS in New Product Innovation

TOPICS IN ALTERNATIVES ASSESSMENT

Pamela J. Spencer, Ph.D. D.A.B.T. VP Regulatory, Product Stewardship & Quality ANGUS Chemical Company

Overview

- What are NAMs
- Types of NAMs
- Are animal tests really the gold standard
- Highlight innovation application strategies



What are NAMs?!

<u>New Approach Methodologies, also known as:</u>

- Predictive Toxicology
- Tox21
- Non-animal alternatives
- Alternative test methods



"adopted as a broadly descriptive reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide <u>information</u> on chemical hazard and risk assessment."¹

¹NIEHS A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States, 2018

Types of NAMs

- I. In silico/computational tools
 - QSARs
 - Machine learning
 - High throughput exposure modeling
- 2. In vitro biological profiling
 - Cell cultures
 - 2, 3-D organotypic culture systems
 - Genomics/transcriptomics
 - Organs on a chip
- 3. Frameworks
 - Adverse Outcome Pathways (AOPs)
 - Defined Approaches (DA)
 - Integrated approaches to testing and assessment (IATA)











A LITTLE MORE DETAIL ON FRAMEWORKS . . .

Adverse Outcome Pathways (AOPs)

An adverse outcome pathway (AOP) is a model that identifies the sequence of biochemical events required to produce a toxic effect when an organism is exposed to a substance.

Skin Sensitization AOP



https://aopwiki.org/wiki/index.php/Aop:40

Defined approaches (DAs) to Testing & Assessment

Relies on:

- Input data generated from identified methods
- A data interpretation procedure, such as machine-learning, flowchart, or decision tree, through which data are evaluated
- For skin sensitization 2 out of 3 approach can be applied (i.e. classified



https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/comptox/ctits/its.html?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=818148

Integrated Approach to Testing & Assessment

 IATAs – are flexible approaches for chemical safety assessment based on the integration and translation of the data derived from multiple methods and sources



Why NAMs? Aren't the Animal Test Adequate?!

NAMs address important challenges in chemical safety assessments ...

- Quicker, lower cost methods (i.e. can evaluate significantly more chemicals for safety than with the current animal tests)
 – Regulatory aspect addressed in next presentation.
- 2. <u>Human relevant</u> information
- 3. Ability to <u>screen chemicals for safety earlier</u> in product development



But Aren't Animal Tests the "Gold Standard"



Application of a Defined Approaches (DAs) to combine in vitro and in silico data using sample decisions trees or machine learning algorithms to predict skin sensitization.

NAMs Outperformed Animal Tests for Predicting Skin Sensitization

All non-animal defined approaches evaluated perform as well or **better** than the mouse at predicting human skin sensitization:

Hazard: 74% (mouse) vs. 75-85% (DAs)

3-class Potency: 59% (mouse) vs. 55-69% (DAs)



Kleinstreuer KC, et. al., Non-animal methods to predict skin sensitization (II): an assessment of defined approaches. Crit Rev Toxicol 2018 May;48(5):359-374

NAMs to Establish Justification for Read-Across to a Chemical Analogue

<u>Background</u>

- Commercial product registered is an isomeric mixture (50:50)
- Want flexibility to vary concentration of individual isomers in product (e.g. 40:60, 80:20)
- R(-) isomer is on inventory list, S(+) isomer is not and would require registration as new chemical
- Can a transcriptomic approach be used to show similar MOA for individual isomers compared to racemic mixture to justify readacross?

Transcriptomic Approach

- Treat relevant cell line(s) with individual isomers & mixture to compare transcriptomic pathways
- Induction of similar cellular responses indicates consistency in MOA



Application of NAMs in New Product Development

Replacement Candidate Computational Toxicology Screen

Endpoint	A	B C1(=0)CCC(C)(C)N1	C C1(=O)CCC(C)(C)N1C	D c1(c)(c)coc(=0)N1	E c1(c)(c)coc(=0)N1c	F cn(c)c(=0)ccc(c)(c)n(c)c
Chemical Structure		× ×			~~~~	o ↓ ,×,
Oral LD50	L: 3911 mkd ¹	l: 2700 mkd ¹	l: 2017 mkd ^{1,2}	vl/l: 5083 mkd ¹	l: 2709 mkd ¹	m: 835 mkd ¹
Inhalation LC50	l: nonhaz ²	m: haz ²	m: haz ²	m: haz ²	m: haz²	l: nonhaz ²
Dermal LD50	vL: >5000 mkd	l: nonhaz ²	l: nonhaz ²	m: haz ²	l: nonhaz ²	l: nonhaz ²
Skin Irritation	M: Irritating	m/h: haz ²	m/h: : haz²	m/h: : haz²	m: haz ²	m: haz ²
Eye Irritation	H: Mod irrit.	h/v̪h: : haz²	h/v̪h: : haz²	h/ỵh: : haz²	h/ <u>yh</u> : : haz²	m: haz²
Skin Sensitization	L: Neg	m: : haz²	l: Neg ²	m: : haz²	l: Neg ²	l: Neg ²
Mutagen	L: Neg ¹	l: Neg ¹	l: Neg ¹	I: Neg ^{1,2}	l: Neg ^{1,2}	l: Neg ¹
Cancer	l: Neg ⁵	l: Neg ⁵	l: Neg ⁵	wh: genotoxic car ⁵	wh: genotoxic car ⁵	l: Neg ⁵
Reprotoxicity	<u>Vh;</u> Repro 1B	m: True ¹	m:True ¹	m: True ¹	m: True ¹	m: True ¹
Acute Ecotox	H: 1.2 mg/L ¹	l/m: 73 mg/L ^{1,2}	l/m: 77 mg/L ^{1,2}	m/h: 8.7 mg/L ^{1,2,3}	m/h: 8.4 mg/L ^{1,2,3}	l/m: 64 mg/L ^{1,2,3}
Chronic Ecotox	l: nonhaz ²	l: nonhaz ²	l: nonhaz ²	l: nonhaz ²	l: nonhaz ²	l: nonhaz ²
Biodegradation	L: Ready bio	m/h: Not ready	m/h: Not ready	m/h: Not ready	m/h: Not ready bio ³	m/h: Not ready
		bio ³	bio ³	bio ³		bio ³
Cramer Class	Intermediate ⁵	Intermediate ⁵	Intermediate ⁵	High⁵	High⁵	Low ⁵

👥 = very low; 📘 = low; М = medium; H = high; 🚜 = very high; Uppercase = test data; lowercase = modeled data

Summary of Results:

- F predicted to have most favorable human health and environment toxicological profile. No significant structural alerts with the exception of
 reproductive toxicity & slower potential to biodegrade. Both should be followed up with further testing to confirm validity of model
 predictions.
- · B and C corrosive to eye, reproductive/developmental, not readily biodegradable
- D and E corrosive to eye, genotoxic carcinogen, reproductive/developmental, acutely toxic to aquatic environment, not readily biodegradable. It is recommended to eliminate both chemicals from further development based on the number of structural alerts.

MODEL USED FOR ASSESSMENT

- ¹ EPA Chem Dashboard, concensus model
- ² UL cheminformatics toolkit model
- ³ EPI Suite BIOWIN
- ⁴ EPI Suite ECOSAR
- ⁵ Toxtree: Modifed Cramer Class or Carcinogenicity decision tree

Food for Thought . . .

- How can NAMs be utilized to advance the field of AA?
- Is there a role for A4 in helping to establish confidence in NAMs?

- Which NAMs and how?

- Should A4 advocate/offer training in NAMs for our members?
- Other opportunities?
- New SOT specialty section, Sustainable Chemicals through Contemporary Toxicology, offers point of partnership with SOT

Development and Use of Predictive Animalfree Toxicology Testing Approaches

TOPICS IN ALTERNATIVES ASSESSMENT

Amy J. Clippinger, Ph.D. PETA International Science Consortium Ltd



Outline

• NAMs

Case studies: Inhalation toxicity testingTraining opportunities

National Academy of Sciences, 2007



TOXICITY TESTING IN THE 21ST CENTURY A VISION AND A STRATEGY



FDA Predictive Toxicology Roadmap, 2017



ICCVAM Strategic Roadmap, 2018

A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States



INTERAGENCY COORDINATING COMMITTEE ON THE VALIDATION OF ALTERNATIVE METHODS

Case Studies: Inhalation Toxicity Testing



- Ventilation rates and breathing mode
- Airway architecture and branching pattern
- Cell type distribution and mucous composition
- Metabolic activity

Illustration modified from Dr. Jack R. Harkema, Professor of Comparative Pathology, Michigan State University









Human Precision Cut Lung Slices (PCLS)

(Co)cultures submerged or grown at the ALI 3D reconstructed human tissues grown at the ALI Microfluidic human lung-on-a-chip

Toxicology in Vitro 48 (2018) 53–70

Case study #1



Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



Toxicology in Vitro 52 (2018) 131–145 Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

Pathway-based predictive approaches for non-animal assessment of acute inhalation toxicity

Amy J. Clippinger^{a,*}, David Allen^b, Holger Behrsing^c, Kelly A. BéruBé^d, Michael B. Bolger^e, Warren Casey^f, Michael DeLorme^g, Marianna Gaça^h, Sean C. Gehenⁱ, Kyle Glover^j, Patrick Hayden^k, Paul Hinderliter^l, Jon A. Hotchkiss^m, Anita Iskandarⁿ, Brian Keyser^o, Karsta Luettichⁿ, Lan Ma-Hock^P, Anna G. Maione^k, Patrudu Makena^o, Jodie Melbourne^a, Lawrence Milchak^g, Sheung P. Ng^q, Alicia Paini^r, Kathryn Page^s, Grace Patlewicz^t, Pilar Prieto^r, Hans Raabe^c, Emily N. Reinke^u, Clive Roper^v, Jane Rose^w, Monita Sharma^a, Wayne Spoo^o, Peter S. Thorne^x, Daniel M. Wilson^m, Annie M. Jarabek^y

Alternative approaches for acute inhalation toxicity testing to address global regulatory and non-regulatory data requirements: An international workshop report

Amy J. Clippinger^{a,*}, David Allen^b, Annie M. Jarabek^c, Marco Corvaro^d, Marianna Gaça^e, Sean Gehen^f, Jon A. Hotchkiss^g, Grace Patlewicz^h, Jodie Melbourne^a, Paul Hinderliterⁱ, Miyoung Yoon^j, Dan Huh^k, Anna Lowit^l, Barbara Buckley^c, Michael Bartels^m, Kelly BéruBéⁿ, Daniel M. Wilson^g, Ian Indans^o, Mathieu Vinken^p





Triethoxysilane (GHS 2, CAS # 998-30-1) Trimethoxysilane (GHS 1, CAS# 2487-90-3),



Methyltrichlorosilane (GHS 3, CAS# 75-79-6),



Trimethylchlorosilane

(GHS 3, CAS# 75-77-4)

BEAS-2B (human bronchial epithelial cell line)



Lactate dehydrogenase (LDH) release
Resazurin metabolism (PrestoBlue®)
Expression of inflammatory markers









Case Study #2

EPA Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel

December 2018



OVS tube: particle size distribution





- Lactate dehydrogenase (LDH) release
- Resazurin metabolism
- Transepithelial electrical resistance



Adverse outcome pathway



Multiple computational and in vitro approaches will be needed to assess the various mechanisms of toxicity following inhalation exposure

Multi-stakeholder collaborations on data sharing and validation efforts foster the development of non-animal approaches that can be used to protect human health without using animals

Training Resources

EPA's List of NAMs



https://www.epa.gov/assessing-and-managing-chemicalsunder-tsca/alternative-test-methods-and-strategies-reduce



provisions for waiving test and reducing

or refining animal use)

Systemic Toxicity

https://ntp.niehs.nih.gov/go/regaccept

402 (1987, revised 2017)

402 (1987, revised 2017)

EU: Accepted via OECD Test Guideline

scientists participated in

drafting and editing the

revised test guideline

IN VITRO METHODS FOR PREDICTING SERIOUS EYE DAMAGE AND IRRITATION



Note: If the substance is not classified as GHS Cat 1 or GHS No Cat, it is likely to be GHS Cat 2. To avoid *in vivo* testing, additional testing may be conducted with *in vitro* methods that allow classification of Cat 2 chemicals in a weight-of-evidence approach. *In vitro* methods, such as the EVEIT, ParCDRA, or similar ones, which address persistence in the absence of severity, may be suitable. For more information on tiered testing strategies for sevinus eye damage and eye intlation, please see: *Organisation* for Economic Co-operation and Development. 2017. Unlikely adapted approach on testing and assessment. (*INIA*) for services eye damage and eye initiation. Ro-S15. Series on Testing and Assessment.

METHOD	PRINCIPLE OF THE TEST	APPLICABILITY DOMAIN	GHS CATEGORISATION
OECD TG 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying () Chemicals Inducing Serious Eye Damage and () Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Test substance is directly applied to cow eyes obtained as by-products from abattoirs. Corneal opacity (measured quantitatively as the amount of sight transmission through the cornea) and permeability (measured quantitatively as the amount of sodium fluorescein dye that passes across the full thickness of the cornea) are measured. Optional histopathology can be conducted for additional information.	Applicable to solids, liquids (including semi-solids, creams, and wates), and mixtures	For the identification of substances causing serious eye damage (GHS Cot 1) and substances not requiring classification for eye imitation or serious eye damage OECD TG 437 training video available at www.youtube.com/ watch?==ElopSKDHB
OECD TG 438: Isolated Chicken Eye (ICE) Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Test substance is directly applied to chicken eyes obtained as by-products from abattoirs. Corneal swelling, opacity, and fluorescein retention are assessed.	Applicable to solids (may be soluble or insoluble in water), liquids, emutsions, and gels	For the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage
DECD TG 460: Fluorescein Leakage (FL) Test Method for Identifying Doular Corrosives and Severe Irritants	Epithelial monolayer Madin-Darby canine kidney (MDCX) cells are cultured on permeable inserts. The test chemical is applied for I minute and then removed; next, the non-doxic highly fluorescent sodium-fluorescent dye is added, and the amount of dye that passes through the cell layer is measured spectrofluorometrically and used to predict taxicity.	Applicable to water-soluble chemicals or mixtures Limitations for coloured or highly viscous substances (predictivity is improved by increasing the number of wash steps) Not applicable to strong acids and bases, cell finatives, or highly unlatile substances	For the identification of substances causing serious eye damage (BHS Cat 1)
OECD TG 451: Short Time Exposure In Vitro Test Method for Identifying () Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Measures cell viability (MIT assay) of SIRC (Statens Seruminstitut Rabbit Cornea) corneal epithelial cells in 96 well plates. As compounds are generally cleared from human eyes in 1 to 2 minutes or rabbit eyes in 3 to 4 minutes, this test requires a 5-minute exposure.	Applicable to test chemicals that are soluble in saline, DMSO, or mineral oil	For the identification of substances causing serious eye damage (GHS Cat 1) and substances not requiring classification for eye irritation or serious eye damage
OECD TG 492: Reconstructed Numan Carnea-like Epithelium (RhCE) Test Nethod for Identifying Chemicals Not Requiring Classification and Labelling for Eye Irritation or Serious Eye Damage (e.g. EpiDcular®)	MatTek's EpiOcular™ tissue is reconstructed from primary human cells, which have been cultured for several days to form a stratified, highly differentiated squamous epithelium morphologically similar to that found in the human comea. The test substance is exposed to the tissue, and cell viability (MTT assay) is used to predict toxicity.	Applicable to substances and mixtures as well as solids, liquids, semi-solids, and waxes	For the identification of substances not requiring classification for eye irritation or serious eye damage
OECD draft T6: Cytosensor Microphysiometer (CM)	A sub-confluent monalayer of mouse L929 cells is exposed to increasing concentrations of the test chemical. The cellular metabolic rate — measured by pH change in the medium (acidification) — is used to predict toxicity.	Applicable to water-soluble chemicals (substances and mixtures) as well as non-soluble solids, viscous chemicals, and suspensions that maintain uniformity during the analysis time	For the identification of substances causing serious eye damage (GHS Cat 1) and substances that are not classified

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Acute Systemic Toxicity

 REACH webinar series: Alternative Approaches to Mammalian Acute Systemic Toxicity Testing (co-hosted by the PETA International Science Consortium, 2015) Click HERE for webinar.

Adverse Outcome Pathways

- Adverse Outcome Pathway (AOP) Learning Channe Click HERE for multiple AOP-related videos.
- Human Toxicology Project Consortium AOP Online Course
 Click HERE for course modules.
- Adverse Outcome Pathways: From Research to Regulation (three-day workshop co-sponsored by the National Toxicology Program Interagency Center for the Evaluation of
 Alternative Toxicological Methods (NICEATM) and the Physicians Committee for Responsible Medicine; 2014)
- Click HERE for video.
- Introduction to Effectopedia (2014)
- Click HERE for video.

Antibodies

- · Bio-Rad: Recombinant antibody webinars
- Click HERE for webinars.
- The Making of Recombinant Anti-idiotypic Antibodies for High Performance in Bioanalytical Assays (hosted by Bio-Rad; 2016)
 Click HERE for webinar.

Databases, In Silico Modeling, and Read-Across

www.piscltd.org.uk/webinars

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About

In Silico / In Vitro Testing Resources

Institute for *In Vitro* Sciences is a nonprofit research and testing laboratory whose offerings include ocular irritation, cytotoxicity, percutaneous absorption, dermal irritation, dermal corrosion, and dermal sensitisation testing. The Institute for *In Vitro* Sciences also has an extensive education and outreach program, including its International Outreach Program.

IONTOX provides *in vitro* toxicology consulting, product development, and laboratory services to the pharmaceutical, cosmetic, chemical, tobacco, and food additive industries. Also involved in research and development of new *in vitro* technologies, IONTOX has developed a multiple organ culture plate linked with micro-fluidics that assesses systemic toxicity.

ScitoVation develops and implements *in vitro* and computational approaches to assess potential health effects of drugs, food ingredients, and chemicals.

VITO (Flemish Institute for Technological Research) is a research and contract testing laboratory that maintains Good Laboratory Practices (GLP)-certified facilities for interlaboratory validation studies.

XCellR8 conducts non-regulatory and regulatory safety testing, including for eye and skin irritation and skin sensitization.

www.piscltd.org.uk/links-resources



Institute for In Vitro Sciences

Advancing Science & Animal Welfare Together

QSAR TOOLBOX

QSAR TOOLBOX

SOECD ECHA





Practical Methods for In Vitro Toxicology Workshop



European Commission's Joint Research Center Summer School on Non-Animal Approaches in Science



Summer School 2020 INNOVATIVE SCIENCE WITHOUT ANIMALS Amy J. Clippinger, PhD Amy JC@PISCLtd.org.uk www.piscltd.org.uk

Respondents – Reflections and Insights



Shari Franjevic, GreenScreen® Program Manager





Lauren Heine, Director of Safer Materials & Data Integrity





Questions?



Announcements



<u>Home</u> About

Program

Registration Abstracts

News Travel & Accommodation

Grants & Awards Sponsorship

11th World Congress on Alternatives and Animal Use in the Life Sciences

3Rs in transition: *from development to application* 23-27 August 2020 | MECC Maastricht – The Netherlands

ABSTRACT SUBMISSION AND REGISTRATION IS NOW OPEN!

Download the official WC11 APP now!



Announcements

Stay tuned for our official announcement

2020 International Symposium on Alternatives Assessment

- Fall 2020
- California





ASSOCIATION FOR THE ADVANCEMENT OF ALTERNATIVES ASSESSMENT



THANK YOU

