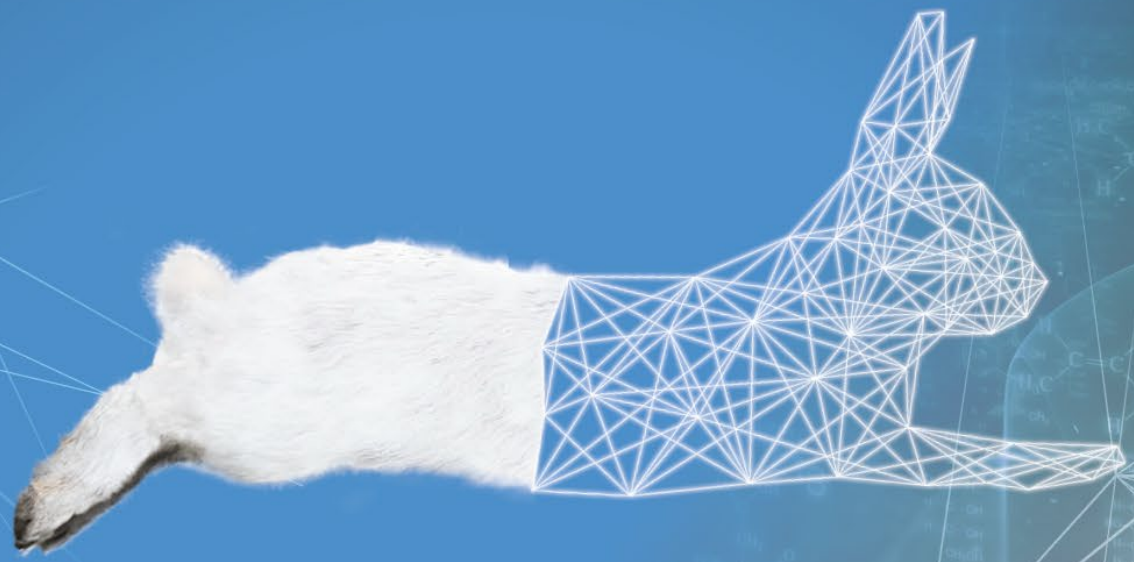
# Acute systemic toxicity testing: Past, present, and future

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

Workshop: Mind the gaps: Prioritizing activities to meet regulatory needs for acute systemic lethality
October 30-31st, 2019



*Toxicol In Vitro*. 2017 June ; 41: 245–259. doi:10.1016/j.tiv.2017.01.004.

# Alternative Approaches for Identifying Acute Systemic Toxicity: Moving from Research to Regulatory Testing

**Jon Hamm[a,*], Kristie Sullivan[b], Amy J. Clippinger[c], Judy Strickland[a], Shannon Bell[a], Barun Bhhatarai[d], Bas Blaauboer[e], Warren Casey[f], David Dorman[g], Anna Forsby[h], Natàlia Garcia-Reyero[i], Sean Gehen[j], Rabea Graepel[k], Jon Hotchkiss[d], Anna Lowit[l], Joanna Matheson[m], Elissa Reaves[l], Louis Scarano[n], Catherine Sprankle[a], Jay Tunkel[o], Dan Wilson[d], Menghang Xia[p], Hao Zhu[q], and David Allen[a]**

# Recommendation #1: Understand the Regulatory Landscape

- What are the acute systemic toxicity testing requirements of global regulatory authorities?
- How are the data used?
- What information is needed?
- What is the path to the acceptance of new methods?

Regulatory Toxicology and Pharmacology 94 (2018) 183–196

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

## Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies

Judy Strickland[a,*], Amy J. Clippinger[b], Jeffrey Brown[b], David Allen[a], Abigail Jacobs[c,1], Joanna Matheson[d], Anna Lowit[e], Emily N. Reinke[f], Mark S. Johnson[f], Michael J. Quinn Jr.[f], David Mattie[g], Suzanne C. Fitzpatrick[h], Surender Ahir[i], Nicole Kleinstreuer[j], Warren Casey[j]

Next Step

➢ Publish international requirements

**Documenting International Acute Systemic Toxicity Testing Requirements to Facilitate Increased Acceptance of Nonanimal Approaches**

Esther Haugabrooks[1], Kristie Sullivan[1]

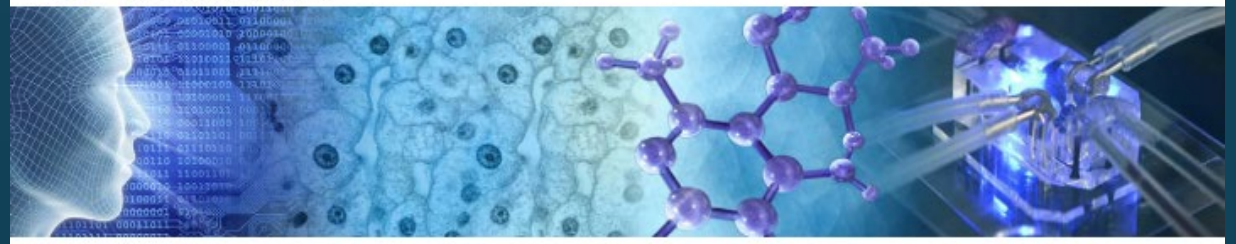
[1]Physicians Committee for Responsible Medicine, Research & Regulatory Affairs

IUTOX 2019

# Recommendation #2: Characterize the Variability of the *In Vivo* Test

NTP National Toxicology Program

https://ntp.niehs.nih.gov/iccvam/meetings/at-models-2018/ppt/4-karmaus.pdf



## Rat Oral Acute Toxicity Database and Evaluation of Variability

Agnes L. Karmaus
Staff Toxicologist, ILS in support of NICEATM

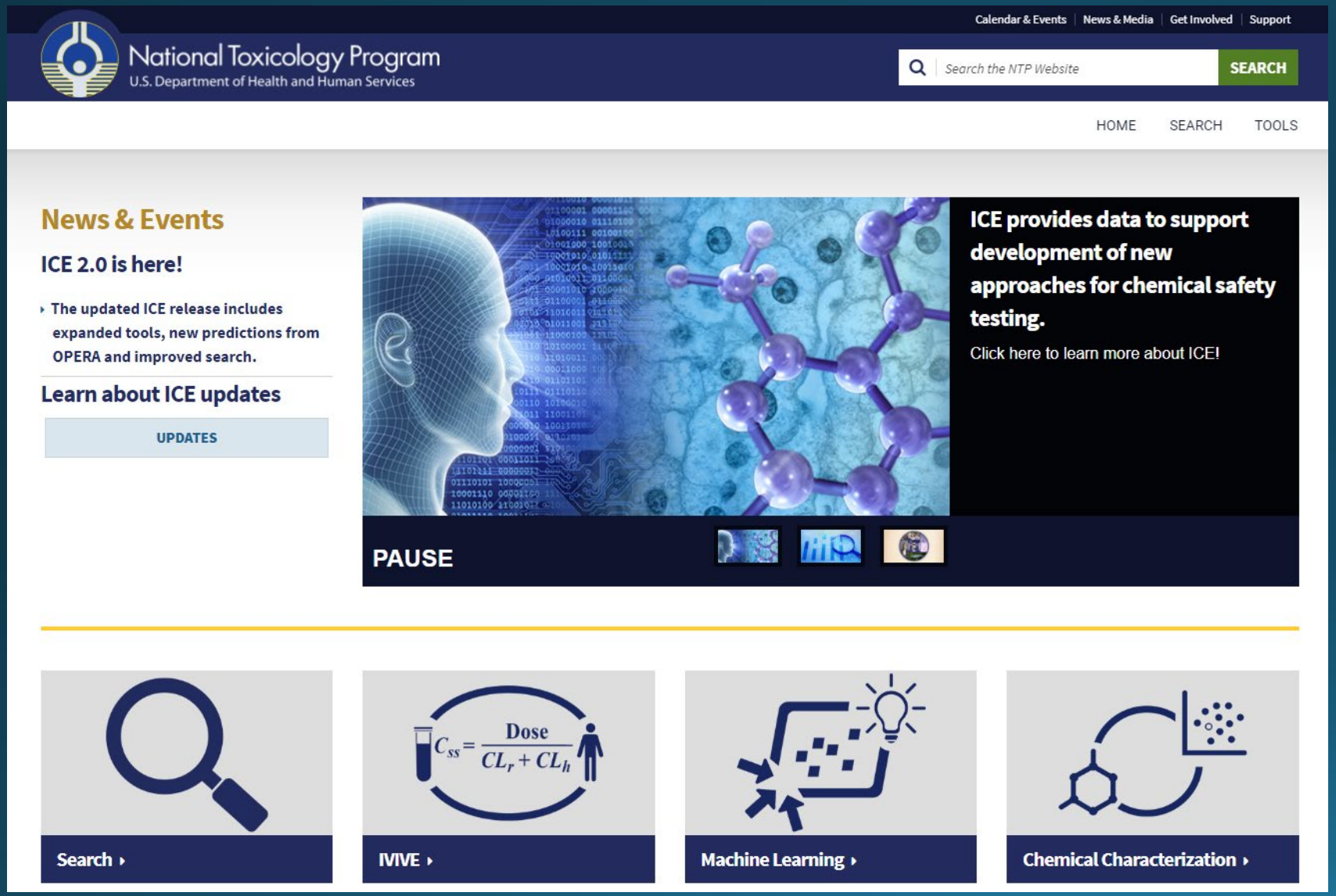
Predictive Models for Acute Oral Systemic Toxicity Workshop
April 11, 2018

*Disclaimer: ILS staff provide technical support for NICEATM, but do not represent NIEHS, NTP, or the official positions of any federal agency.*

Next Step

- Finalize and publish analysis

# Recommendation #3: Compile and Make Available Curated Data




***Webinar***: Dr. Shannon Bell
July 31, 2019
The Integrated Chemical Environment: Tools and Data to Support Toxicity Assessments
www.piscltd.org.uk/training-videos-webinars

Next Step
- Curate additional data

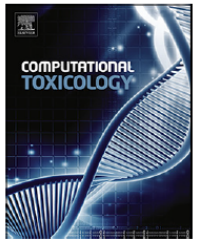
# Recommendation #4: Develop *In Silico* Models

Computational Toxicology 8 (2018) 21–24

Contents lists available at ScienceDirect

Computational Toxicology

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




## Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation

Nicole C. Kleinstreuer[a], Agnes L. Karmaus[b], Kamel Mansouri[b], David G. Allen[b], Jeremy M. Fitzpatrick[c], Grace Patlewicz[c,*]

Next steps

- Define when the acute oral model can be used/accepted
- Acute inhalation toxicity:
  - CASE Ultra (MultiCASE) and Cheminformatics Suite (Underwriters)
  - Do sufficient data exist in ICE to develop a model for acute inhalation toxicity?

# Recommendation #5: Form Work Group to Discuss Acute Inhalation Toxicity

Toxicology in Vitro 48 (2018) 53–70

Contents lists available at ScienceDirect

Toxicology in Vitro

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

## 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 Hinderliter[i], Miyoung Yoon[j], Dan Huh[k], Anna Lowit[l], Barbara Buckley[c], Michael Bartels[m], Kelly BéruBé[n], Daniel M. Wilson[g], Ian Indans[o], Mathieu Vinken[p]

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. Gehen[i], Kyle Glover[j], Patrick Hayden[k], Paul Hinderliter[l], Jon A. Hotchkiss[m], Anita Iskandar[n], Brian Keyser[o], Karsta Luettich[n], 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]

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018

DOI: 10.1089/aivt.2018.0004

# Prevalidation of an Acute Inhalation Toxicity Test Using the EpiAirway *In Vitro* Human Airway Model

George R. Jackson, Jr., Anna G. Maione, Mitchell Klausner, and Patrick J. Hayden

Toxicology Letters 316 (2019) 119–126

Contents lists available at ScienceDirect

Toxicology Letters

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

## Exposure of 19 substances to lung A549 cells at the air liquid interface or under submerged conditions reveals high correlation between cytotoxicity *in vitro* and CLP classifications for acute lung toxicity

Katrin Gohlsch[a], Harald Mückter[a], Dirk Steinritz[a,b], Michaela Aufderheide[c], Sebastian Hoffmann[d], Thomas Gudermann[a], Andreas Breit[a,*]

Toxicology in Vitro 58 (2019) 245–255

Contents lists available at ScienceDirect

Toxicology in Vitro

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

## Validation of the CULTEX® Radial Flow System for the assessment of the acute inhalation toxicity of airborne particles

Amelie Tsoutsoulopoulos[a,*], Katrin Gohlsch[b], Niklas Möhle[c], Andreas Breit[b], Sebastian Hoffmann[e], Olaf Krischenowski[c,d], Harald Mückter[b], Thomas Gudermann[b], Horst Thiermann[a], Michaela Aufderheide[c,d], Dirk Steinritz[a,b]

## *In Vitro* Approach for Assessing Respiratory Toxicity in Human Lung Cells

Monita Sharma[1], Sandra Verstraelen[2], Evelien Frijns[2], Frederick Maes[2], Amy J. Clippinger[1]
[1]PETA International Science Consortium Ltd., UK
[2]VITO, Flemish Institute for Technological Research, Belgium

Approaches to efficiently and effectively assess the toxicity of chemicals on the human respiratory tract using *in vitro* systems would provide useful information to inform product development and risk management decisions. Presented here is an approach to help better understand the appropriate *in vitro* system to use and the biological markers to monitor based on the test chemical under evaluation. In this study, BEAS-2B cells (a human bronchial epithelial cell line) were exposed to various concentrations of

Mark Higuchi, US EPA, ORD
Use of a Novel In Vitro Exposure System with Various Human Primary Bronchial Epithelial Cell Cultures to Assess Respiratory Toxicity

**FIFRA Scientific Advisory Panel**
**Meeting Minutes and Final Report**
**No. 2019-01**

**Peer Review on Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)**

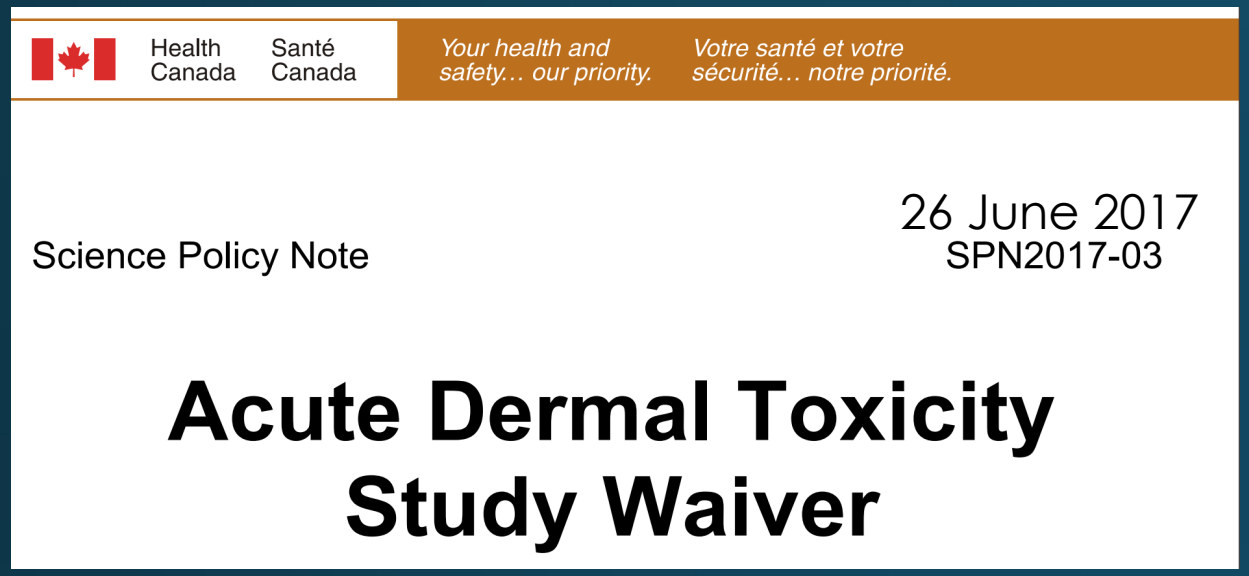
**December 4 and 6, 2018**
**FIFRA Scientific Advisory Panel Meeting**

21 Webinars
- IATA
- In Silico
- In Vitro

www.piscltd.org.uk/inhalation-webinars

Next steps: Ongoing research and discussions
Focus on systemic effects

# Recommendation #6: Waivers for Acute Dermal Toxicity Tests



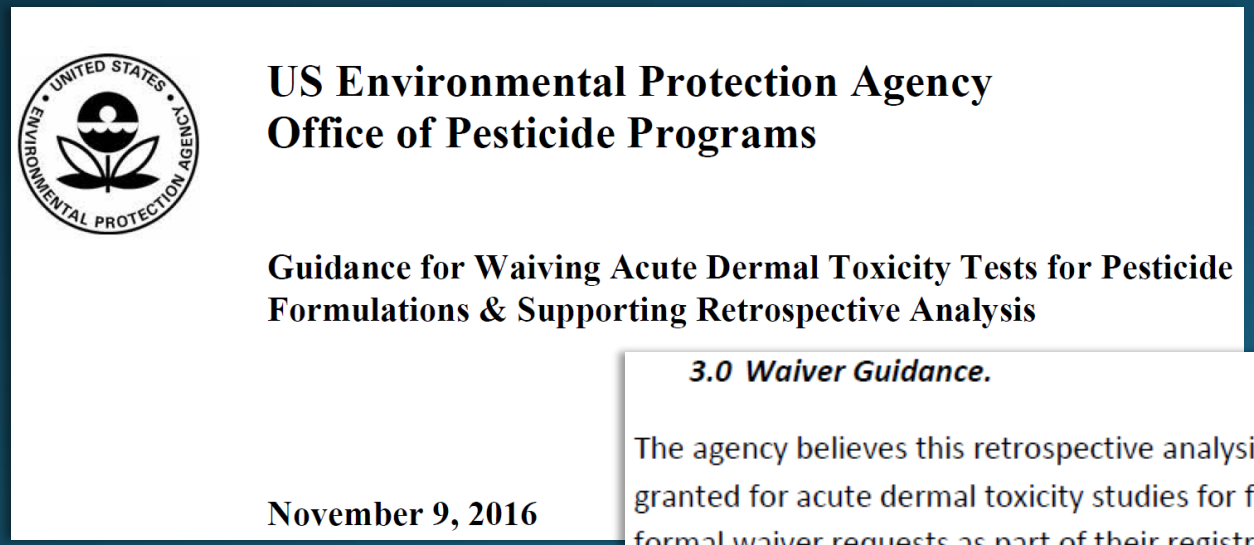
Health Canada Santé Canada

*Your health and safety… our priority.* *Votre santé et votre sécurité… notre priorité.*

Science Policy Note

26 June 2017
SPN2017-03

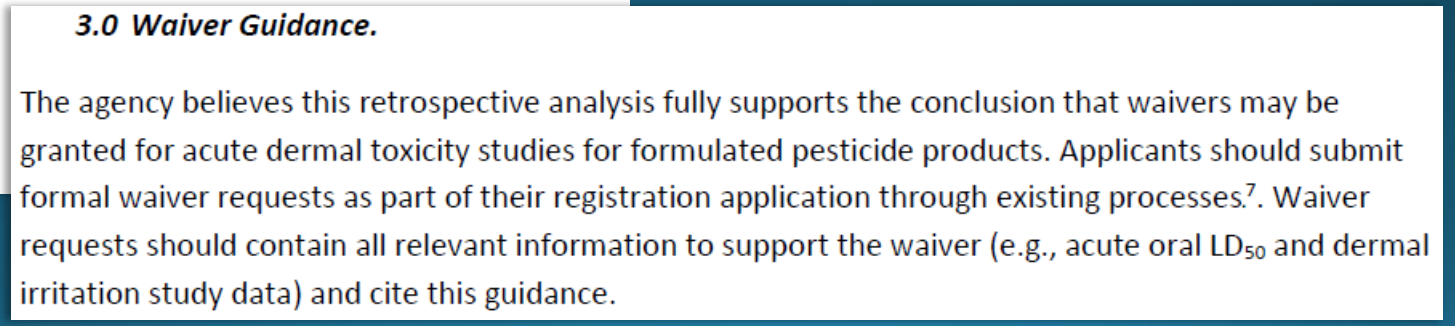
**Acute Dermal Toxicity Study Waiver**



**US Environmental Protection Agency**
**Office of Pesticide Programs**

**Guidance for Waiving Acute Dermal Toxicity Tests for Pesticide Formulations & Supporting Retrospective Analysis**

**November 9, 2016**



***3.0 Waiver Guidance.***

The agency believes this retrospective analysis fully supports the conclusion that waivers may be granted for acute dermal toxicity studies for formulated pesticide products. Applicants should submit formal waiver requests as part of their registration application through existing processes[7]. Waiver requests should contain all relevant information to support the waiver (e.g., acute oral $LD_{50}$ and dermal irritation study data) and cite this guidance.

- Waivers accepted by some agencies
- Need for international acceptance

# Recommendation #7: Develop Criteria for Use of the GHS Mixtures Equation

Regulatory Toxicology and Pharmacology 82 (2016) 99–110

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

## GHS additivity formula: A true replacement method for acute systemic toxicity testing of agrochemical formulations

M. Corvaro [a, *], S. Gehen [b], K. Andrews [a], R. Chatfield [a], C. Arasti [a], J. Mehta [a]

Regulatory Toxicology and Pharmacology 92 (2018) 407–419

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

## GHS additivity formula: can it predict the acute systemic toxicity of agrochemical formulations that contain acutely toxic ingredients?

Andrew Van Cott[a,*], Charles E. Hastings[a], Robert Landsiedel[b], Susanne Kolle[b], Stefan Stinchcombe[b]

3.4.2013 EN Official Journal of the European Union L 93/85

**COMMISSION REGULATION (EU) No 284/2013**

**of 1 March 2013**

**setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market**

EPA United States Environmental Protection Agency

www.epa.gov/pesticide-registration/mixtures-equation-pilot-program-reduce-animal-testing

Environmental Topics | Laws & Regulations | About EPA | Search EPA.gov

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**Pesticide Registration**

- Pesticide Registration Home
- About Pesticide Registration
- Electronic Submission of Applications
- Pesticide Registration Manual
- Fees and Waivers
- Registration Information by Type of Pesticide

### Mixtures Equation Pilot Program to Reduce Animal Testing

Under EPA's initiative to develop non-animal alternatives for acute toxicity testing (see Letter to Stakeholders on EPA Office of Pesticide Programs' Goal to Reduce Animal Testing), we are announcing the start of a pilot program to evaluate the usefulness and acceptability of a mathematical tool (the GHS Mixtures Equation), which is used in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS). The goal of the pilot is to evaluate the utility and acceptability of the GHS Mixtures Equation as an alternative to animal oral and inhalation toxicity studies for pesticide formulations.

- Equation is accepted in the EU
- Need for international acceptance
  - In-country analysis and criteria for use

# Recommendation #8: Understanding the Mechanisms of Acute Toxicity

*ALTEX. 2019;36(1):39-64.*

Review Article

## Investigating Cell Type Specific Mechanisms Contributing to Acute Oral Toxicity

*Pilar Prieto, Rabea Graepel, Kirsten Gerloff, Lara Lamon, Magdalini Sachana, Francesca Pistollato, Laura Gribaldo, Anna Bal-Price and Andrew Worth*

EU Commission Joint Research Centre (JRC), Ispra, Italy

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018

DOI: 10.1089/aivt.2017.0040

## Development of a Profiler for Facile Chemical Reactivity Using the Open-Source Konstanz Information Miner

Sanjeeva J. Wijeyesakere, Daniel M. Wilson, Raja Settivari, Tyler R. Auernhammer, Amanda K. Parks, and M. Sue Marty

APPLIED IN VITRO TOXICOLOGY
Volume 4, Number 2, 2018

DOI: 10.1089/aivt.2017.0041

## Profiling Acute Oral and Inhalation Toxicity Data Using a Computational Workflow to Screen for Facile Chemical Reactivity

Dan Wilson, Sanjeeva J. Wijeyesakere, Amanda K. Parks, Tyler R. Auernhammer, Shannon Krieger, Jon A. Hotchkiss, and M. Sue Marty

# Recommendation #9: Education and Training on *In Vitro* and *In Silico* Approaches

- on use and data interpretation
- for early-career scientists, industry users, and regulatory reviewers
- in the form of conference sessions, focused seminars, webinars, and workshops

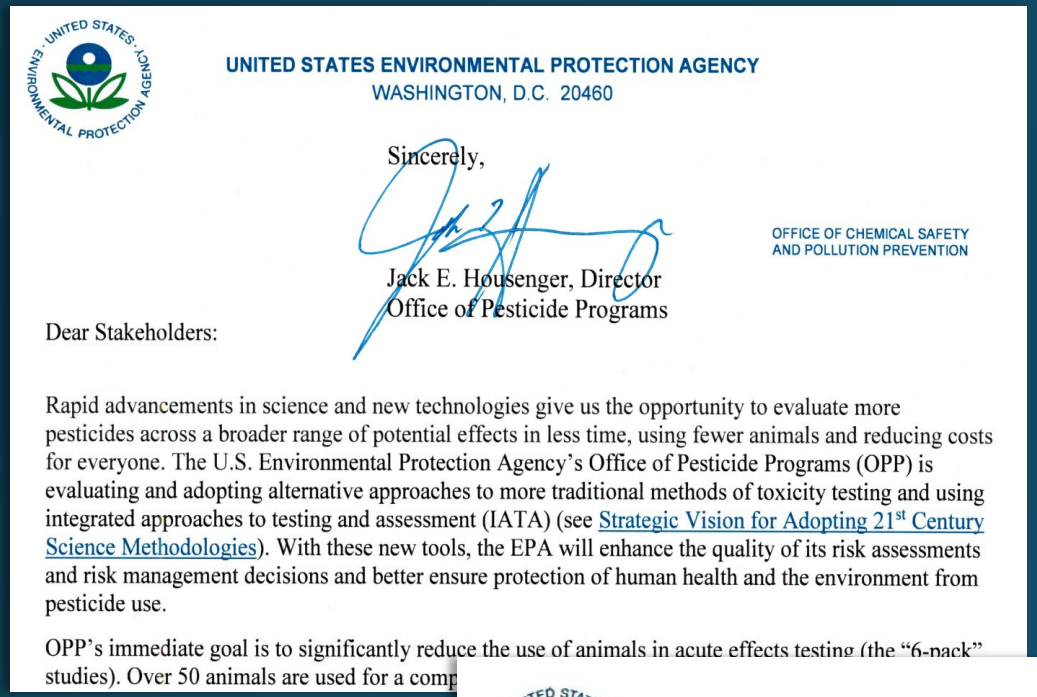

*Webinar Series on the Use of NAMs in Risk Assessment*
*Co-organized by the PETA-ISC, US EPA, and PCRM*
**Webinar 4: New Approaches for Fish Toxicity Testing**
13 November 2019 11:30am – 1:00pm ET
David Volz, Ph.D, University of California, Riverside
Michelle Embry, Health and Environmental Sciences Institute
www.piscltd.org.uk/nam-webinars

Annual early-career scientist award to attend the Institute for In Vitro Sciences' Practical Methods for In Vitro Toxicology Workshop, sponsored by the PETA International Science Consortium Ltd.


**Society of Toxicology Continuing Education Course (CE-08)**
**In Vitro Approaches to Assess the Toxicity of Inhaled Substances**
Sunday, March 15, 2020; 1:15-5pm
- David Allen, Integrated Laboratory Systems, Inc.
- Arno Gutleb, Luxembourg Institute of Science and Technology
- Holger Behrsing, Institute for In Vitro Sciences
- Monique Perron, U.S. EPA Office of Pesticide Programs


**Lessons Learned and Practical Considerations for the Use of *In Vitro* Exposure Systems to Assess Respiratory Toxicity**
- Lawrence Milchak, 3M Company
- Sandra Verstraelen, VITO
- Mark Higuchi, US EPA Office of Research and Development
- Vicki Stone, Heriot Watt University
- Holger Behrsing, Institute for In Vitro Sciences

# Recommendation #10: Leadership



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Sincerely,

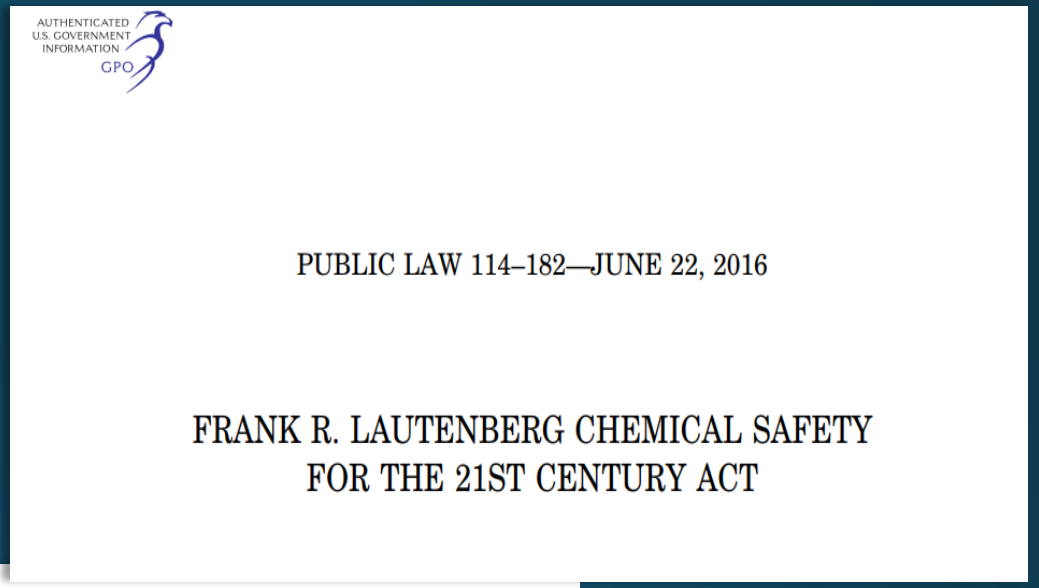
OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Jack E. Housenger, Director
Office of Pesticide Programs

Dear Stakeholders:

Rapid advancements in science and new technologies give us the opportunity to evaluate more pesticides across a broader range of potential effects in less time, using fewer animals and reducing costs for everyone. The U.S. Environmental Protection Agency's Office of Pesticide Programs (OPP) is evaluating and adopting alternative approaches to more traditional methods of toxicity testing and using integrated approaches to testing and assessment (IATA) (see Strategic Vision for Adopting 21st Century Science Methodologies). With these new tools, the EPA will enhance the quality of its risk assessments and risk management decisions and better ensure protection of human health and the environment from pesticide use.

OPP's immediate goal is to significantly reduce the use of animals in acute effects testing (the "6-pack" studies). Over 50 animals are used for a comp



AUTHENTICATED
U.S. GOVERNMENT
INFORMATION
GPO

PUBLIC LAW 114–182—JUNE 22, 2016

FRANK R. LAUTENBERG CHEMICAL SAFETY
FOR THE 21ST CENTURY ACT

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 10, 2019

THE ADMINISTRATOR

**MEMORANDUM**

**SUBJECT:** Directive to Prioritize Efforts to Reduce Animal Testing

**FROM:** Andrew R. Wheeler
Administrator

Amy J. Clippinger, Ph.D.

AmyJC@PISCLtd.org.uk

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