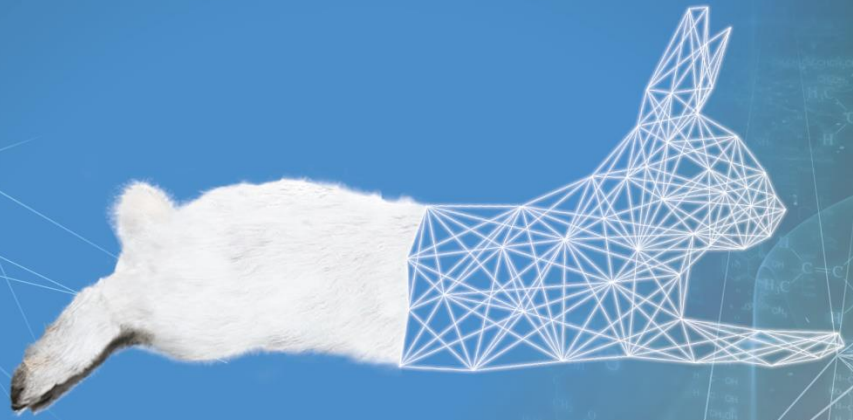
Establishing Scientific Confidence in Reliable and Relevant Testing Approaches

Amy J. Clippinger, Ph.D.
PETA Science Consortium International e.V.



PETA SCIENCE CONSORTIUM INTERNATIONAL e.V.

FUNDING

TRAINING, WORKSHOPS, WEBINARS

DATA ANALYSIS

PUBLICATIONS AND PRESENTATIONS

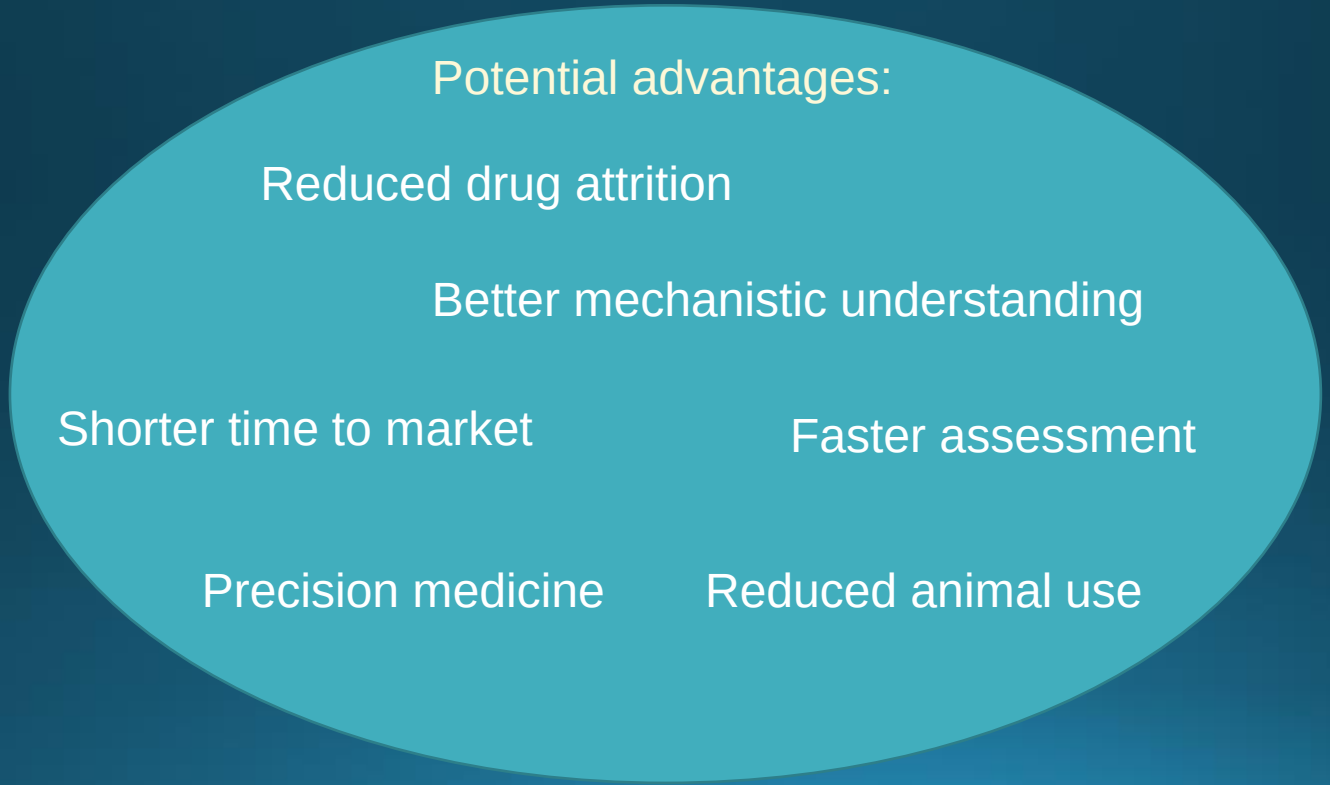
STANDARDS MAKING ORGANISATIONS

Outline

- Current regulatory landscape and interest in new approaches
- Framework to establish scientific confidence in new approaches
  - Example: Eye irritation
  - Example: Respiratory toxicity

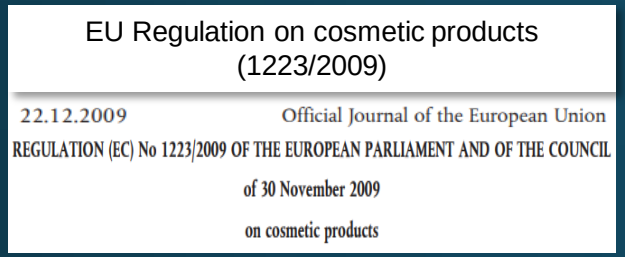
Regulatory Landscape

Substantial interest and investment in advancing the development of non-animal testing approaches, driven by a desire for better protection of human health and the environment as well as ethical, time, and monetary considerations



Legislation that requires, or strongly encourages, the replacement of animal testing

EU Regulation on cosmetic products (1223/2009)



22.12.2009 Official Journal of the European Union

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 30 November 2009

on cosmetic products

EU Directive on the protection of animals used for scientific purposes (2010/63)

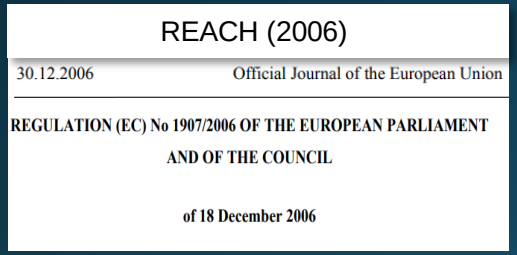
20.10.2010 EN Official Journal of the European Union L 276/33

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

REACH (2006)



30.12.2006 Official Journal of the European Union

REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 18 December 2006

EU Regulation of plant protection products, 2009

24.11.2009 EN Official Journal of the European Union L 309/1

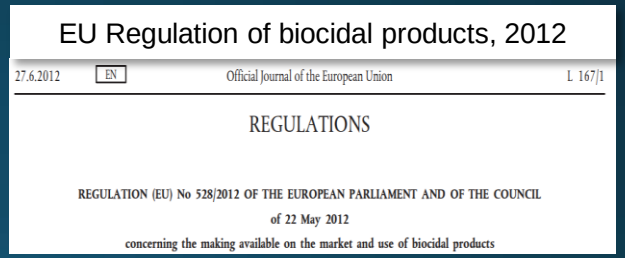
REGULATIONS

REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 21 October 2009

concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

EU Regulation of biocidal products, 2012



27.6.2012 EN Official Journal of the European Union L 167/1

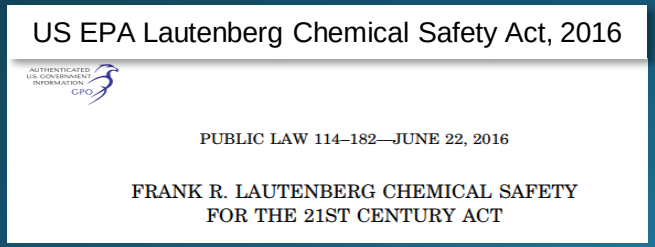
REGULATIONS

REGULATION (EU) No 528/2012 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 May 2012

concerning the making available on the market and use of biocidal products

US EPA Lautenberg Chemical Safety Act, 2016



AUTHENTICATED U.S. GOVERNMENT INFORMATION GPO

PUBLIC LAW 114–182—JUNE 22, 2016

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

US FDA Modernization Act, 2022

AUTHENTICATED U.S. GOVERNMENT INFORMATION GPO

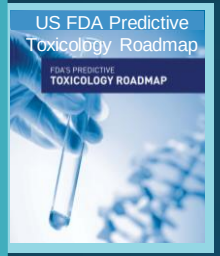
117TH CONGRESS 2D SESSION

S. 5002

To allow for alternatives to animal testing for purposes of drug and biological product applications.

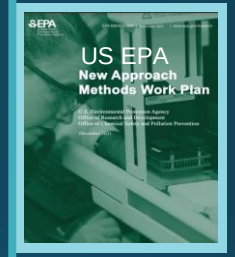
2007

2016



2017

2018



2020

2022

Ongoing

ICCVAM and EPA Confidence Building Framework

ECHA Roadmap

Framework to Establish Scientific Confidence in New Approaches

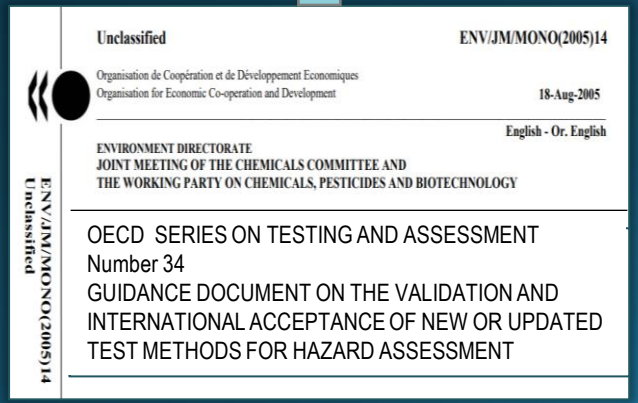
Archives of Toxicology (2022) 96:2865–2879
https://doi.org/10.1007/s00204-022-03365-4

REVIEW ARTICLE

A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]

[1] PETA Science Consortium International e.V.
[2] European Commission, Joint Research Center
[3] Organisation for Economic Co-Operation and Development, Hazard Assessment and Pesticides Programmes
[4] National Institutes of Health, DNTP, National Institutes of Environmental Health Sciences
[5] US Consumer Product Safety Commission
[6] US Environmental Protection Agency, Office of Pollution Prevention and Toxics
[7] National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
[8] US Environmental Protection Agency, Office of Pesticide Programs



Unclassified ENV/JM/MONO(2005)14

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development

18-Aug-2005

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

OECD SERIES ON TESTING AND ASSESSMENT
Number 34
GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT

ENV/JM/MONO(2005)14
Unclassified

Arch Toxicol (2018) 92:611–617
https://doi.org/10.1007/s00204-017-2097-4

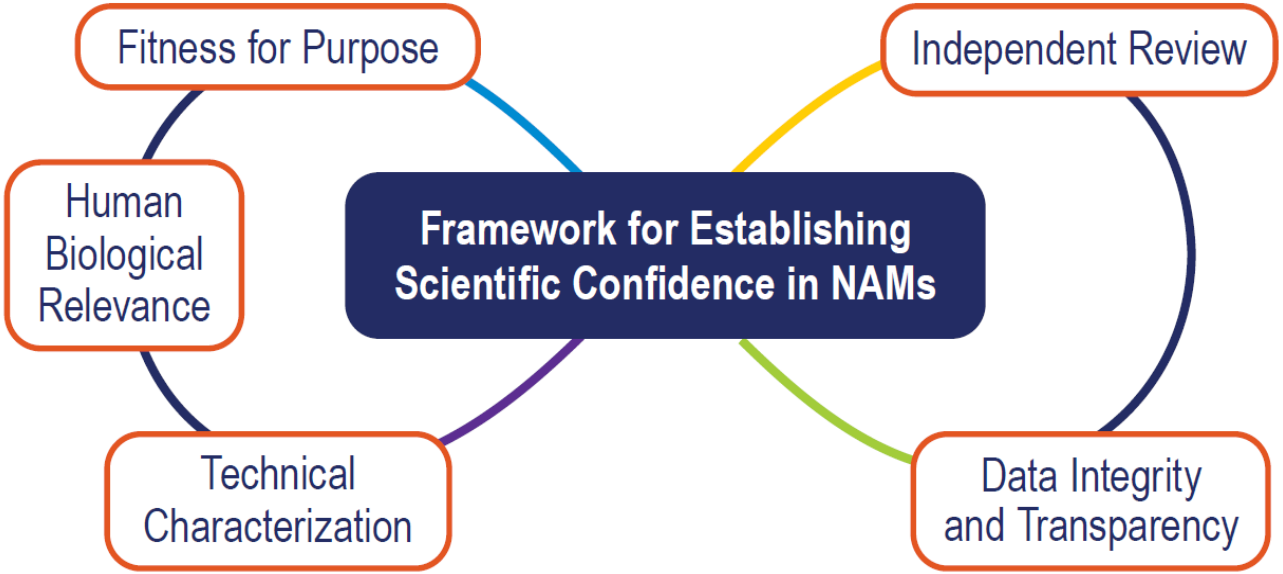
REGULATORY TOXICOLOGY

Standardisation of defined approaches for skin sensitisation testing to support regulatory use and international adoption: position of the International Cooperation on Alternative Test Methods

S. Casati[1] · K. Aschberger[1] · J. Barroso[1] · W. Casey[2] · I. Delgado[3] · T. S. Kim[4] · N. Kleinstreuer[2] · H. Kojima[5] · J. K. Lee[4] · A. Lowit[6] · H. K. Park[4] · M. J. Régimbald-Krnel[7] · J. Strickland[8] · M. Whelan[1] · Y. Yang[9] · Valérie Zuang[1]

A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]



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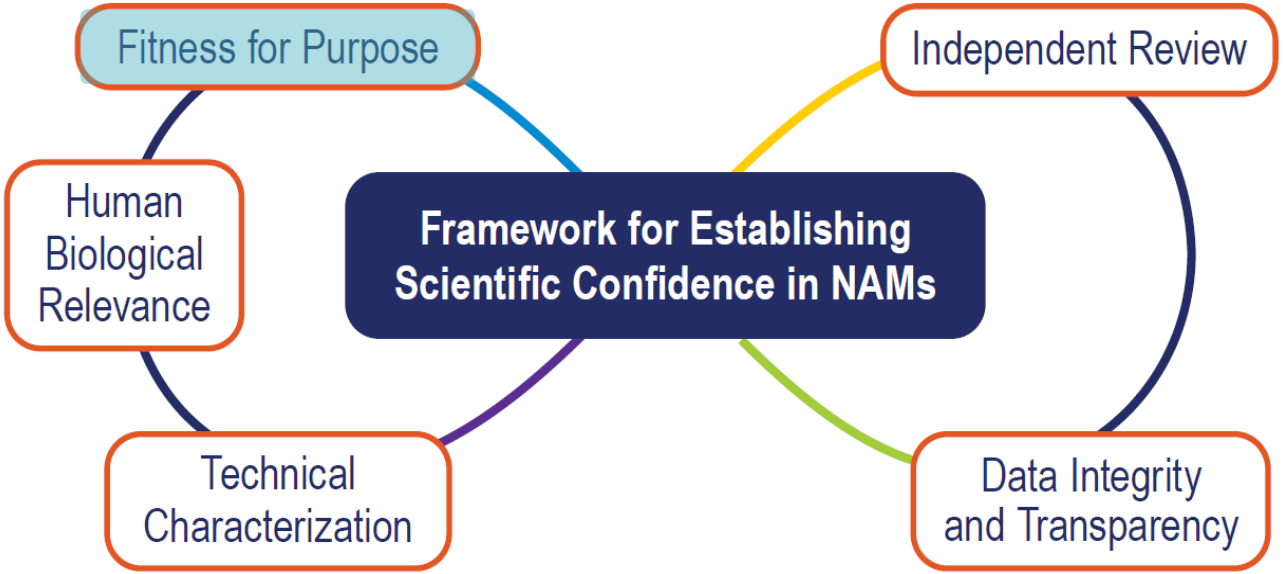

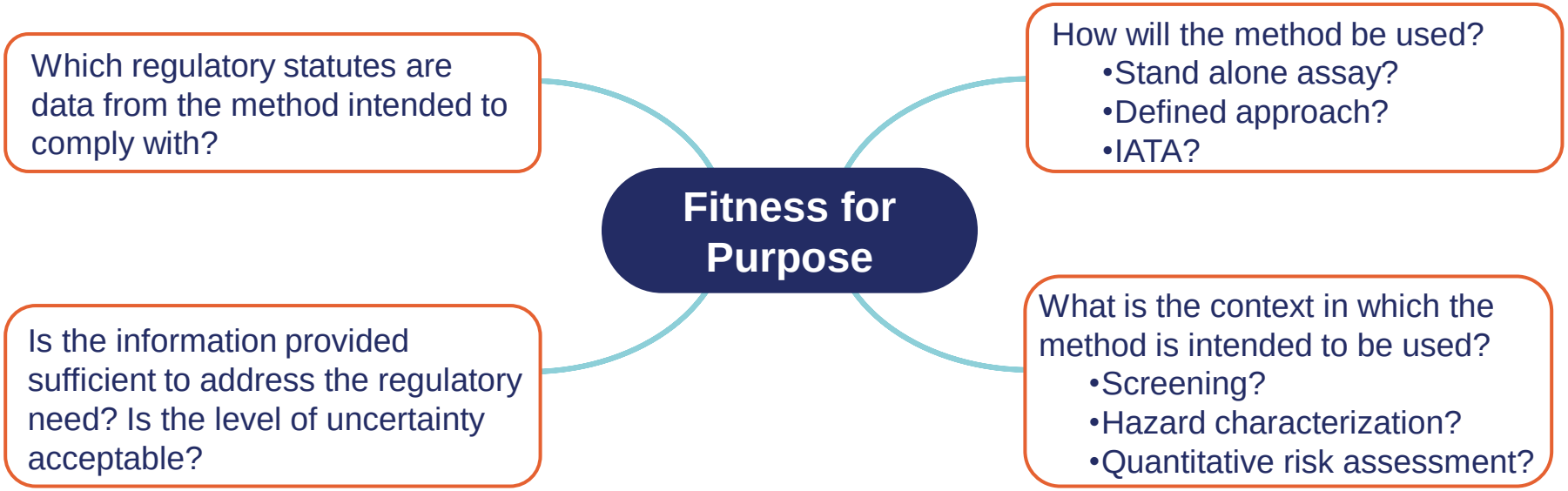
Fitness for Purpose
Which regulatory statutes are data from the method intended to comply with?
How will the method be used?
•Stand alone assay?
•Defined approach?
•IATA?
Is the information provided sufficient to address the regulatory need? Is the level of uncertainty acceptable?
What is the context in which the method is intended to be used?
•Screening?
•Hazard characterization?
•Quantitative risk assessment?

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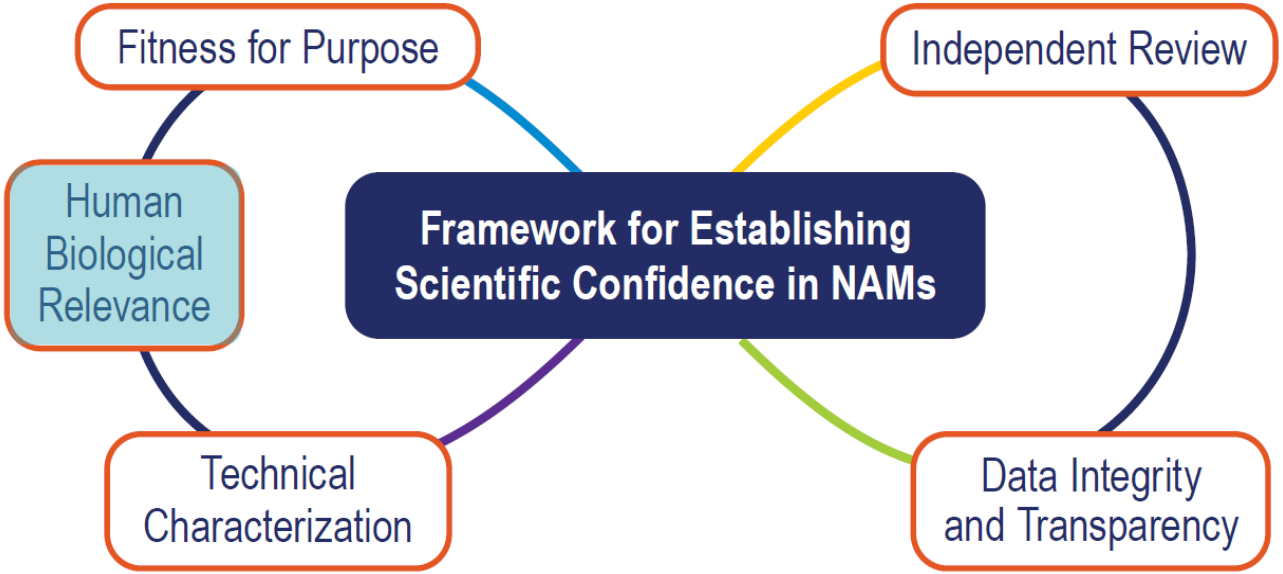

Human Biological Relevance

Concordance with human responses, when high quality human data are available

Similarities between the physiology of or biology measured by the test system, and human biology (i.e., does the method capture key aspects of human biology or mechanisms of toxicity?)
•Consider cell types used, the structure of the target organ, incorporation of human dosimetry modelling

A framework for establishing scientific confidence in new approach methodologies

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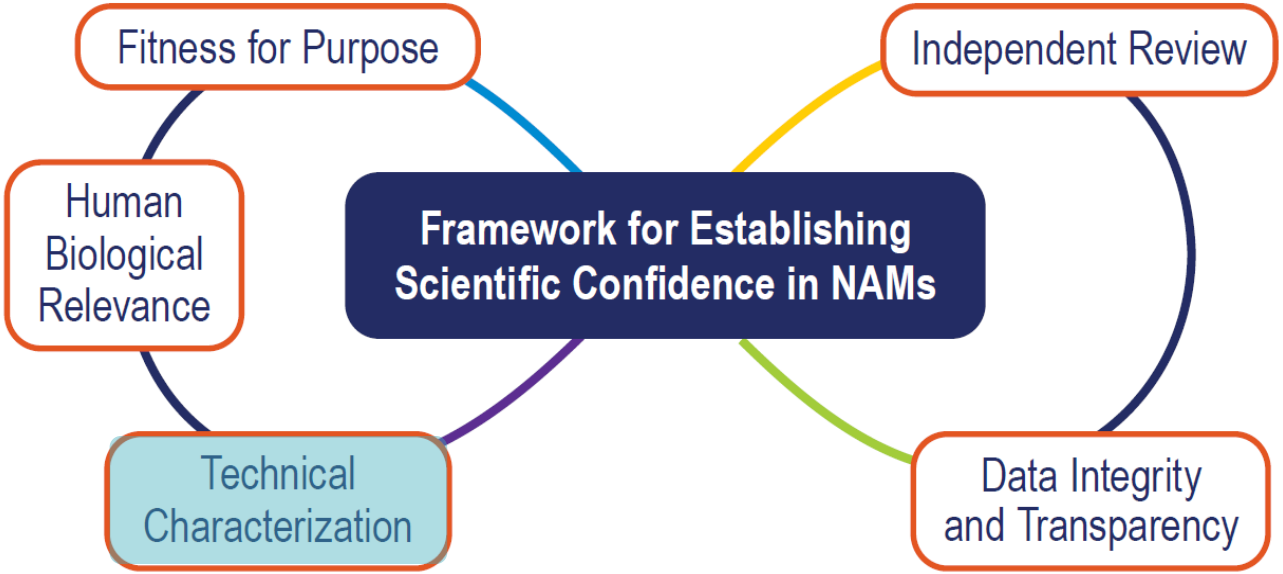


Technical Characterization

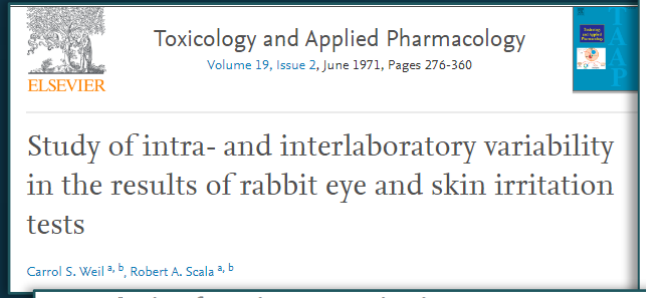
Evaluate:
- accuracy
- intra-laboratory reproducibility
- transferability
- applicability domain
- reference chemicals and controls
- limits of detection and quantification

What is considered acceptable may depend on the method's intended use

Data reporting should allow for evaluation of the method, including:
- protocol
- equipment
- computational models

Accuracy

While historically determined by directly comparing the results from a new method to results from traditional animal test methods, this should not be the default way to determine accuracy

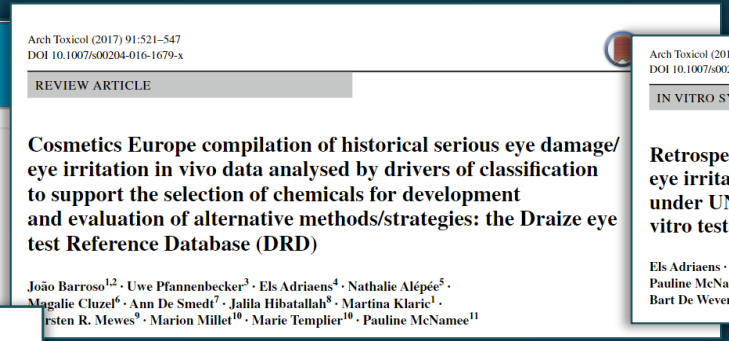


Toxicology and Applied Pharmacology
Volume 19, Issue 2, June 1971, Pages 276-360

ELSEVIER

Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests

Carrol S. Weil [a, b], Robert A. Scala [a, b]

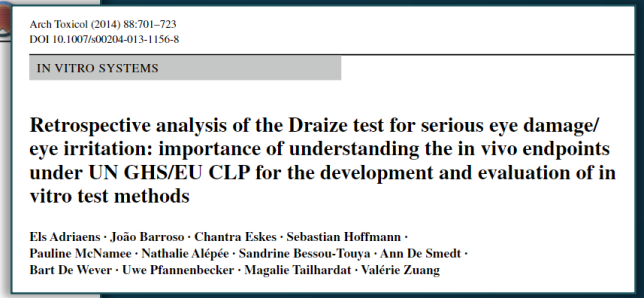


Arch Toxicol (2017) 91:521–547
DOI 10.1007/s00204-016-1679-x

REVIEW ARTICLE

Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD)

João Barroso[1,2] · Uwe Pfannenbecker[3] · Els Adriaens[4] · Nathalie Alépée[5] · Magalie Cluzel[6] · Ann De Smedt[7] · Jalila Hibatallah[8] · Martina Klaric[1] · Kirsten R. Mewes[9] · Marion Millet[10] · Marie Templier[10] · Pauline McNamee[11]

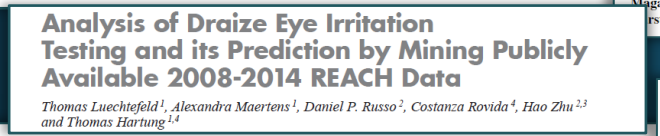


Arch Toxicol (2014) 88:701–723
DOI 10.1007/s00204-013-1156-8

IN VITRO SYSTEMS

Retrospective analysis of the Draize test for serious eye damage/eye irritation: importance of understanding the in vivo endpoints under UN GHS/EU CLP for the development and evaluation of in vitro test methods

Els Adriaens · João Barroso · Chantra Eskes · Sebastian Hoffmann · Pauline McNamee · Nathalie Alépée · Sandrine Bessou-Touya · Ann De Smedt · Bart De Wever · Uwe Pfannenbecker · Magalie Tailhardat · Valérie Zuang



Analysis of Draize Eye Irritation Testing and its Prediction by Mining Publicly Available 2008-2014 REACH Data

Thomas Luechtefeld[1], Alexandra Maertens[1], Daniel P. Russo[2], Costanza Rovida[4], Hao Zhu[2,3] and Thomas Hartung[1,4]

Regulatory Toxicology and Pharmacology 122 (2021) 104920

Contents lists available at ScienceDirect

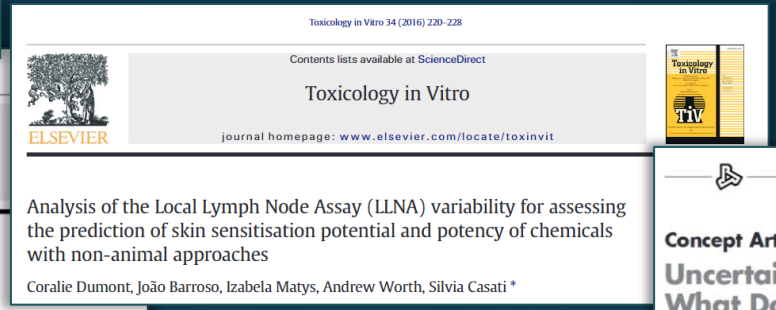
Regulatory Toxicology and Pharmacology

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

ELSEVIER

Analysis of variability in the rabbit skin irritation assay

John P. Rooney[a,*], Neepa Y. Choksi[a], Patricia Ceger[a], Amber B. Daniel[a], James Truax[a], David Allen[a], Nicole Kleinstreuer[b]



Toxicology in Vitro 34 (2016) 220–228

Contents lists available at ScienceDirect

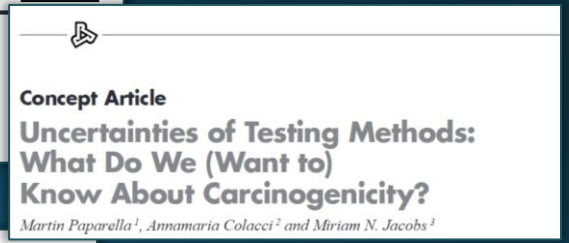
Toxicology in Vitro

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

ELSEVIER

Analysis of the Local Lymph Node Assay (LLNA) variability for assessing the prediction of skin sensitisation potential and potency of chemicals with non-animal approaches

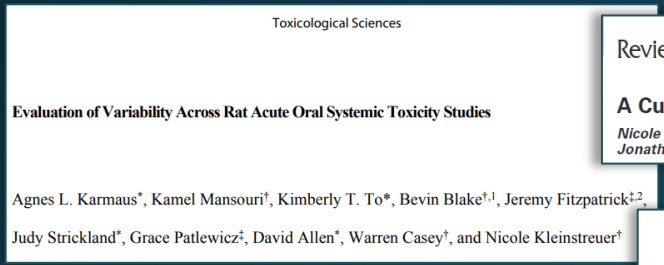
Coralie Dumont, João Barroso, Izabela Matys, Andrew Worth, Silvia Casati *



Concept Article

Uncertainties of Testing Methods: What Do We (Want to) Know About Carcinogenicity?

Martin Paparella[1], Annamaria Colacci[2] and Miriam N. Jacobs[3]



Toxicological Sciences

Evaluation of Variability Across Rat Acute Oral Systemic Toxicity Studies

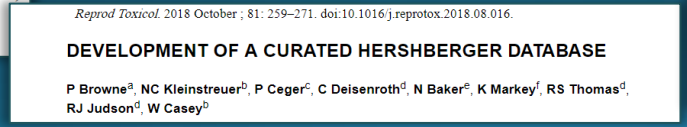
Agnes L. Karmaus*, Kamel Mansouri†, Kimberly T. To*, Bevin Blake†,1, Jeremy Fitzpatrick‡,2, Judy Strickland*, Grace Patlewicz‡, David Allen*, Warren Casey†, and Nicole Kleinstreuer†

Review

A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1510183.

A Curated Database of Rodent Uterotrophic Bioactivity

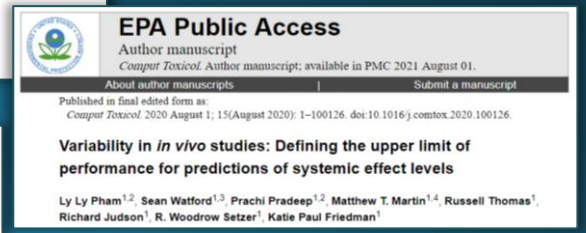
Nicole C. Kleinstreuer,[1] Patricia C. Ceger,[1] David G. Allen,[1] Judy Strickland,[1] Xiaoqing Chang,[1] Jonathan T. Hamm,[1] and Warren M. Casey[2]



Reprod Toxicol. 2018 October ; 81: 259–271. doi:10.1016/j.reprotox.2018.08.016.

DEVELOPMENT OF A CURATED HERSHBERGER DATABASE

P Browne[a], NC Kleinstreuer[b], P Ceger[c], C Deisenroth[d], N Baker[e], K Markey[f], RS Thomas[d], RJ Judson[d], W Casey[b]



EPA Public Access
Author manuscript
Comput Toxicol. Author manuscript; available in PMC 2021 August 01.

About author manuscripts | Submit a manuscript

Published in final edited form as:
Comput Toxicol. 2020 August 1; 15(August 2020): 1–100126. doi:10.1016/j.comtox.2020.100126.

Variability in *in vivo* studies: Defining the upper limit of performance for predictions of systemic effect levels

Ly Ly Pham[1,2], Sean Watford[1,3], Prachi Pradeep[1,2], Matthew T. Martin[1,4], Russell Thomas[1], Richard Judson[1], R. Woodrow Setzer[1], Katie Paul Friedman[1]

Accuracy

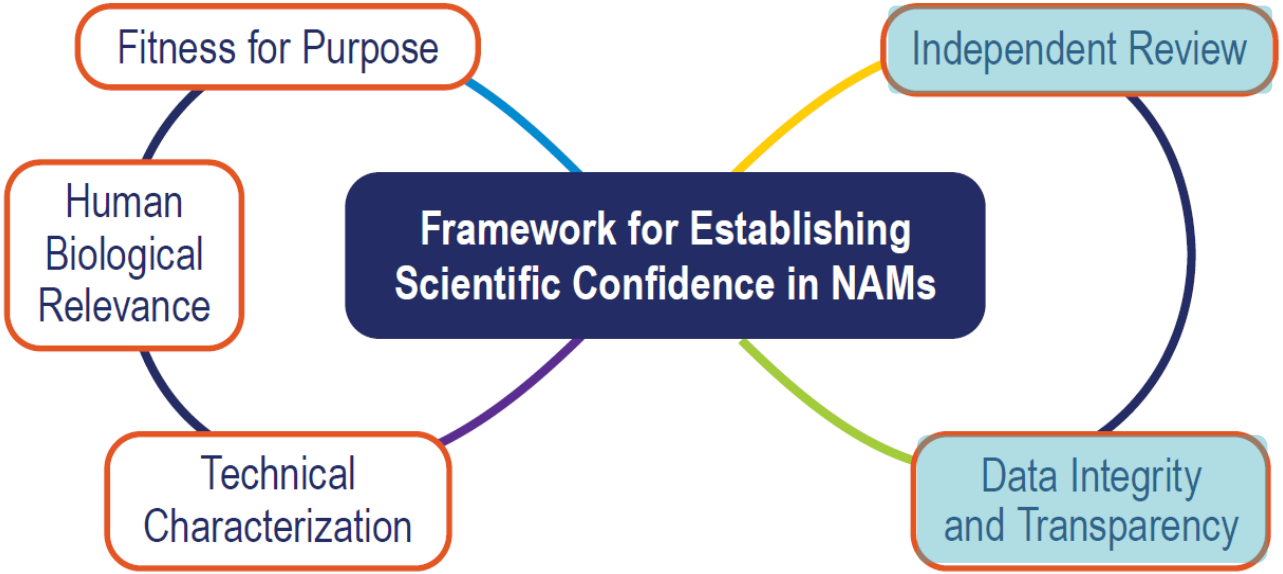
Animal test methods cannot be assumed to be reproducible or provide data relevant to human biology, and therefore, should they should not be the default reference method for determining accuracy of another method

Accuracy can be addressed through

- demonstrating biological relevance and reproducibility
- comparisons across reliable and relevant methods
- correct identification of positive and negative reference chemicals derived from human data

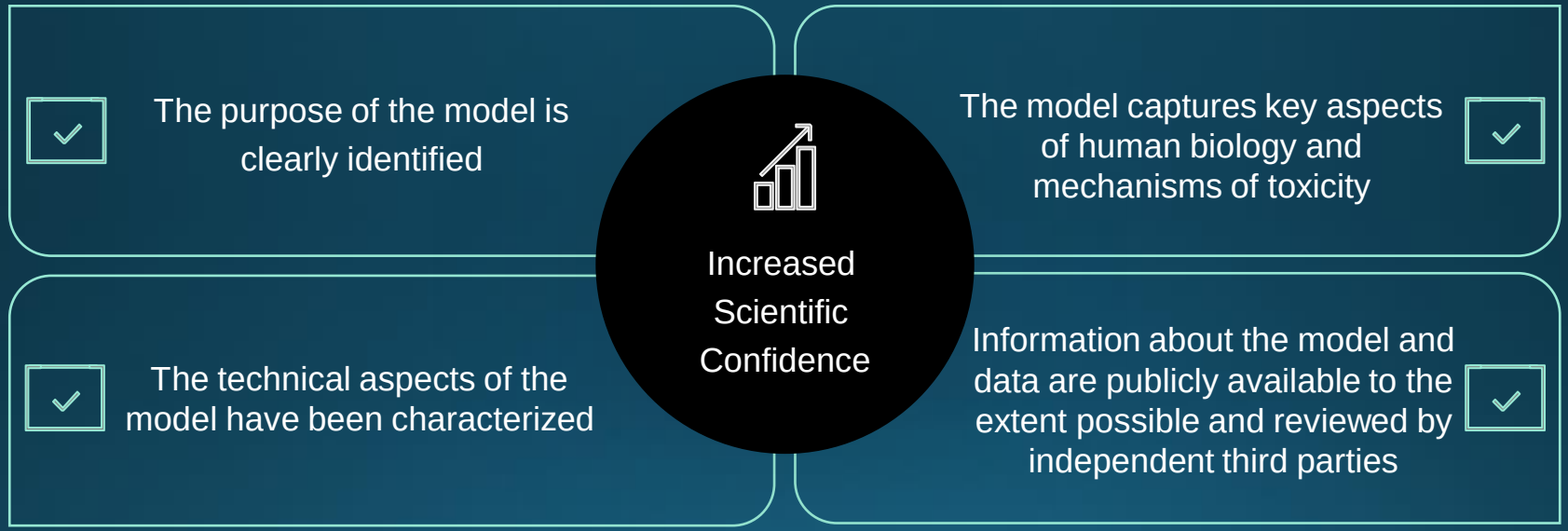
A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]



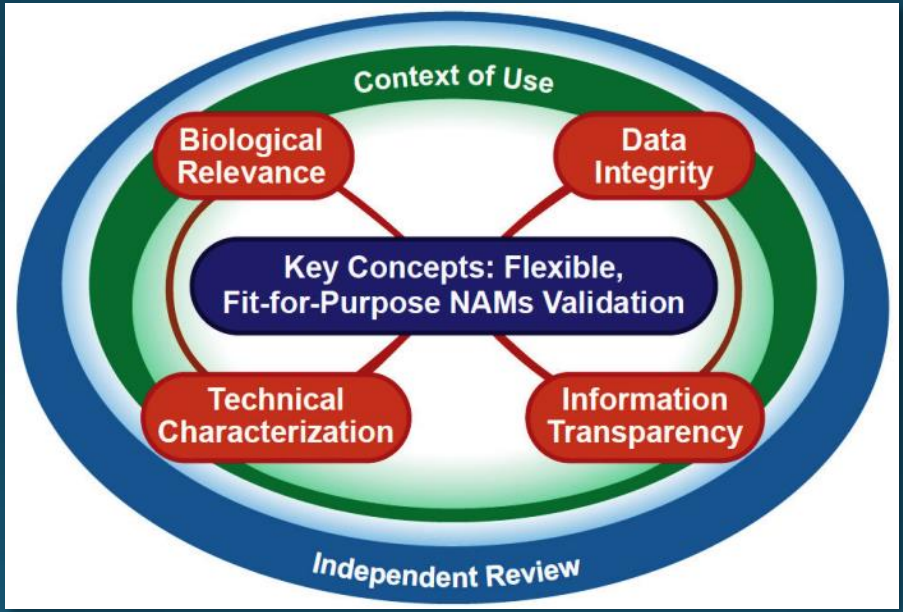
- Information about a new approach should be transparently communicated and undergo independent scientific review:
  - Raw data
  - How to interpret data
  - Information related to fitness for purpose, relevance to humans, and technical characterization

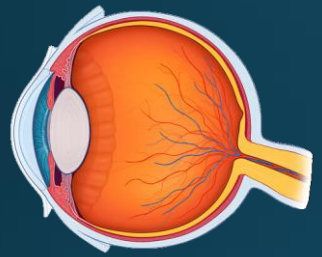
- The appropriate level of review will be determined by agency



Fitness for purpose • Human biological relevance • Technical characterization • Data integrity and transparency • Independent review

ICCVAM Validation Workgroup: Scientific Confidence Framework





Eye Irritation and Corrosion

Application of the Framework to the Agrochemical Sector

Retrospective Analysis: 232 agrochemical formulations

Prospective *In Vitro/Ex Vivo* Testing: 29 agrochemical formulations

Formulations and existing data donated by companies

CUTANEOUS AND OCULAR TOXICOLOGY
2021, VOL. 40, NO. 2, 145–167
https://doi.org/10.1080/15569527.2021.1910291

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Human-relevant approaches to assess eye corrosion/irritation potential of agrochemical formulations

Amy J. Clippinger[a], Hans A. Raabe[b], David G. Allen[c], Neepa Y. Choksi[c], Anna J. van der Zalm[a], Nicole C. Kleinstreuer[d], João Barroso[e] and Anna B. Lowit[f]

[a]PETA Science Consortium International e.V., Stuttgart, Germany; [b]Institute for In Vitro Sciences, Inc., Gaithersburg, MD, USA; [c]Integrated Laboratory Systems, LLC, Research Triangle Park, NC, USA; [d]National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [e]European Commission, Joint Research Centre (JRC), Ispra, VA, Italy; [f]US Environmental Protection Agency Office of Pesticide Programs, Washington, DC, USA

- Paper reviews the existing methods for eye irritation with a focus on reproducibility and human relevance

All of our publications can be accessed at www.thepsci.eu/scipubs

Human Biological Relevance: Draize Rabbit Eye Test

There are numerous biological differences between rabbit and human eyes, including:

- rabbits have a nictitating membrane; humans do not
- rabbits have a larger conjunctival sac than humans
- the tissue structure, thickness, and biochemistry of human and rabbit cornea differ
- rabbits produce less tears than humans
- the pH of a rabbit eye aqueous humor is more alkaline (8.2) than that of a human eye (7.1-7.2)

Reproducibility: Draize Rabbit Eye Test

- Data submitted to the European Chemicals Agency
- 491 substances with at least 2 Draize eye tests
- Conditional probabilities of Draize evaluations based on a previous test result

Prior GHS category	1	2A	2B	NC
1 (serious eye damage)	73%	16%	0%	10%
2A (irritant)	4%	33%	4%	59%
2B (mild irritant)	0%	4%	16%	80%
NC (non-irritant)	1%	4%	2%	94%

Adapted from Luechtefeld et al., ALTEX 33(2), 2016.

Defined Approaches to Classify Agrochemical Formulations into EPA Hazard Categories: Case Study using EpiOcular™ Reconstructed Human Corneal Epithelium and Bovine Corneal Opacity and Permeability Assays

Anna J. van der Zalm[1], Amber B. Daniel[2], Hans A. Raabe[3], Neepa Choksi[2,*], Tara Flint Silva[4], Julie Breeden-Alemi[4], Lindsay O'Dell[5], Nicole C. Kleinstreuer[6], Anna B. Lowit[5], David G. Allen[2], Amy J. Clippinger[1]

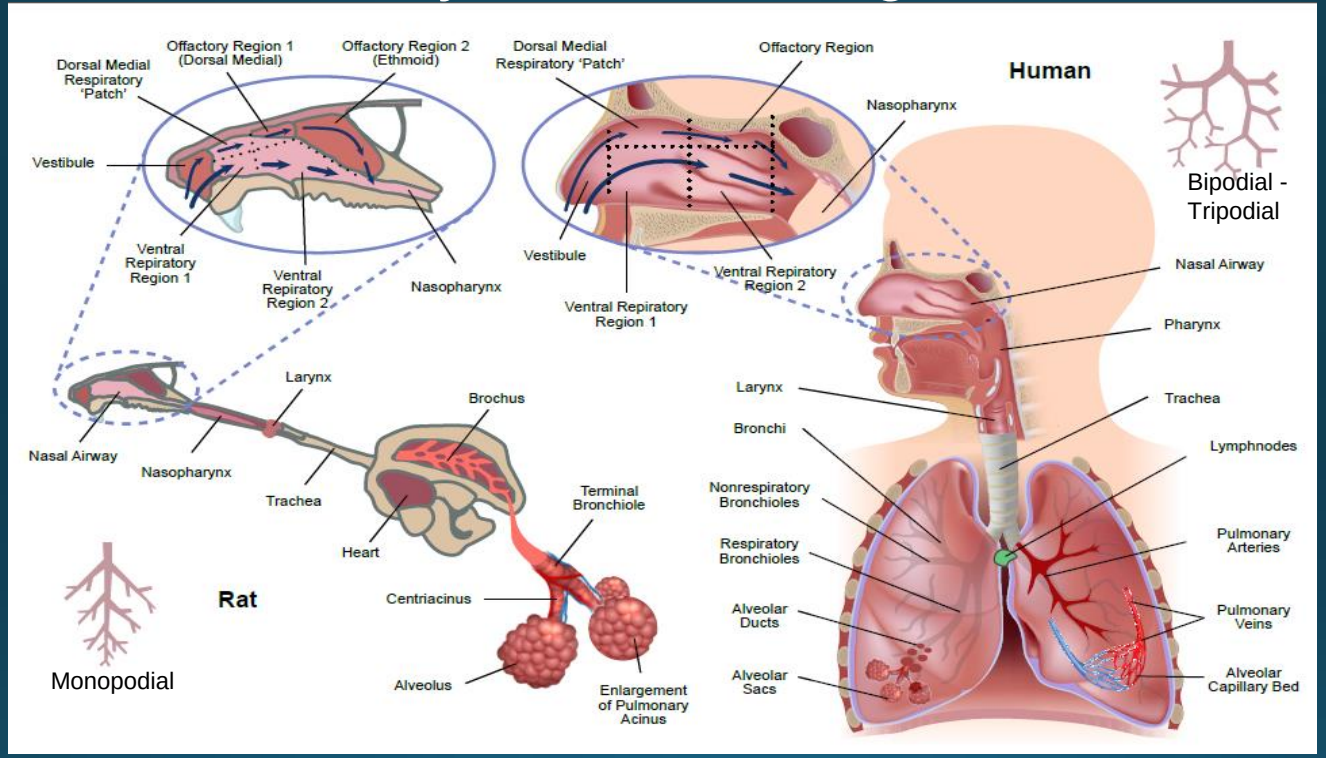
[1]PETA Science Consortium International e.V.; [2]Inotiv; [3]Institute for In Vitro Sciences; [4]US Environmental Protection Agency Office of Pesticide Programs; [5]US Environmental Protection Agency Office of Pollution Prevention and Toxics; [6]NICEATM

- *Fitness for Purpose*: Methods can be used for classifying agrochemical formulations into EPA hazard categories.
- *Human Biological Relevance*: The methods reflect key aspects of human biology and capture key mechanisms of irritation in humans.
- *Technical characterization*: The two methods are OECD test guidelines and have haven been extensively and transparently characterized. They have a greater reproducibility than the *in vivo* rabbit test.

➢ There is high scientific confidence in the use of these two approaches for assessing the eye irritation potential of agrochemical formulations.

Inhalation Toxicity

Inhalation Toxicity: Human Biological Relevance



- 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, Michigan State University

Differences in the Biology of the Human and Rat Respiratory Tracts and its Impact on Toxicological Assessment, Manuscript in Preparation.

Acute Inhalation Toxicity: Reproducibility

NICEATM rat acute inhalation toxicity database

Sources of information:

- ECHA REACH, ChemIDplus, US Department of Defense, US Environmental Protection Agency, NIOSH Pocket Guide
- 1025 chemicals passed quality assurance

Database will be used to:

- Conduct reproducibility analysis
- Build predictive models, similar to the CATMoS project (LC50, Hazard Categories (GHS, EPA, CPSC, DOT), Binary (Toxic vs Non-toxic))

NICEATM = National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods

Conclusions

Framework provides a streamlined and consistent way to help us incorporate advancements in toxicological tools for assessing human health effects

- allows us to evaluate the limitations and advantages of new and existing test methods
- allows us to address the question of whether a new method is ‘as good as or better than’ an existing test method based on reliability, relevance, and fitness for purpose
- demonstrates why new methods should not be expected to produce the same results as an existing test method

Amy J. Clippinger, PhD

AmyJC@thePSCI.eu

www.thePSCI.eu

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Jeff Brown

Franziska Grein