



Alternative Toxicity Testing: North American Agriculture Industry

Angela Hofstra
August 24, 2018

Classification: PUBLIC

Protecting Human Health

- Predictive; efficient; accepted
- Where we are
- Where are headed
- Where we need help



Where we are now





- Jobs ▾
- Immigration ▾
- Travel ▾
- Business ▾
- Benefits ▾
- Health ▾
- Taxes ▾
- More services ▾

Home → Health Canada → About Health Canada → Branches and Agencies

Pest Management Regulatory Agency

The Health Canada [Pest Management Regulatory Agency](#) (PMRA) is responsible for pesticide regulation in Canada. Created in 1995, this branch of Health Canada consolidates the resources and responsibilities for pest management regulation.

Pesticides are stringently regulated in Canada to ensure they pose minimal risk to human health and the environment. Under authority of the *Pest Control Products Act*, Health Canada:



Office of Chemical Safety and Pollution Prevention (OCSPP)

202-564-2902

[About the Office of Chemical Safety and Pollution Prevention](#)

[OCSPP Organization Chart](#)

- [Federal Insecticide, Fungicide, and Rodenticide Act \(FIFRA\)](#)
- [Federal Food, Drug and Cosmetic Act \(FFDCA\)](#)
- [Toxic Substances Control Act \(TSCA\)](#)
- [Pollution Prevention Act](#), and
- portions of other statutes (see below).

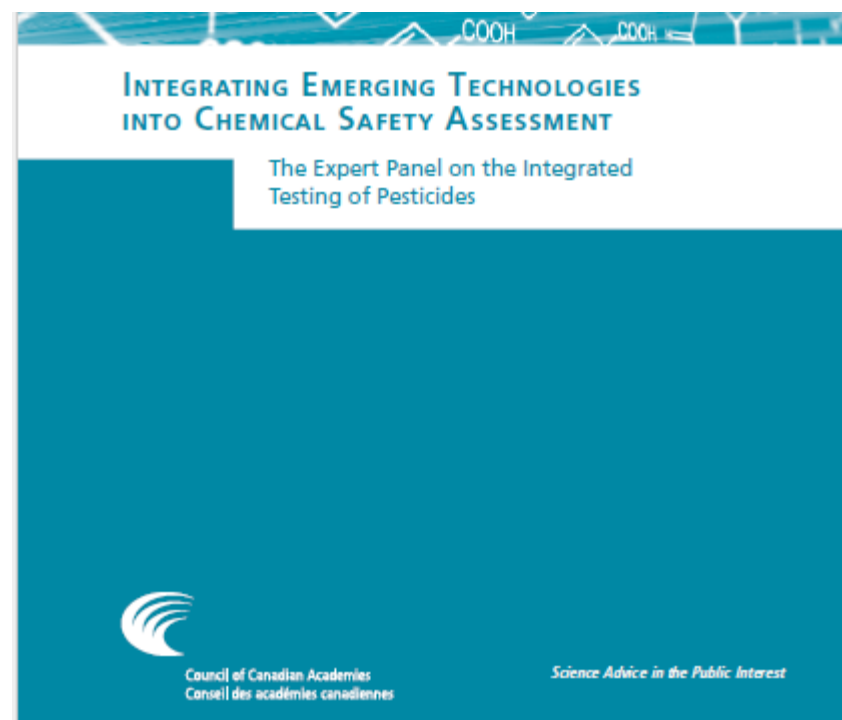
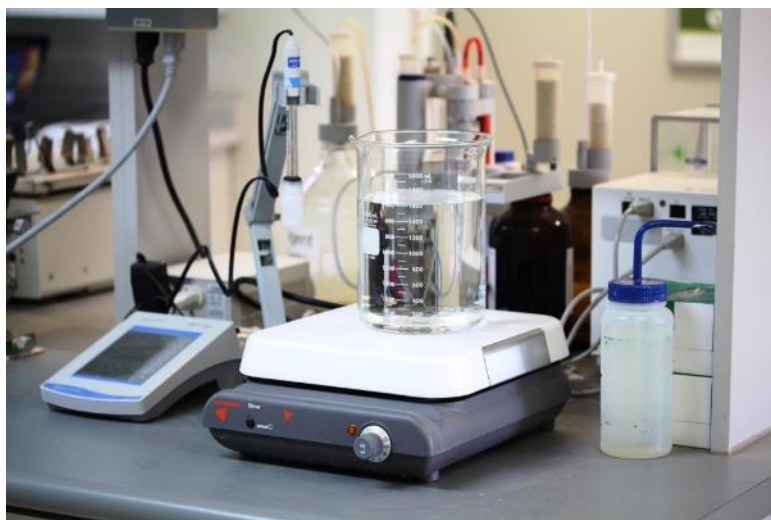
The Office of Chemical Safety and Pollution Prevention includes:

- [Office of Pesticide Programs](#)
- [Office of Pollution Prevention and Toxics](#)
- [Office of Science Coordination and Policy](#)



Process for Establishing & Implementing Alternative Approaches to Traditional *In Vivo* Acute Toxicity Studies

EPA's Office of Pesticide Programs (OPP) has developed a strategic vision for implementing the 2007 NRC report on Toxicity Testing in the 21st Century. This strategic vision has multiple components involving a combination of computational and predictive modeling approaches, *in vitro* techniques, and limited, targeted *in vivo* testing, to supplement or replace the existing toxicity tests required in 40 CFR part 158 in support of pesticide registration.





Science Policy Note

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



Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides

Pest Management Regulatory Agency
Health Evaluation Directorate



Office of Pesticide Programs

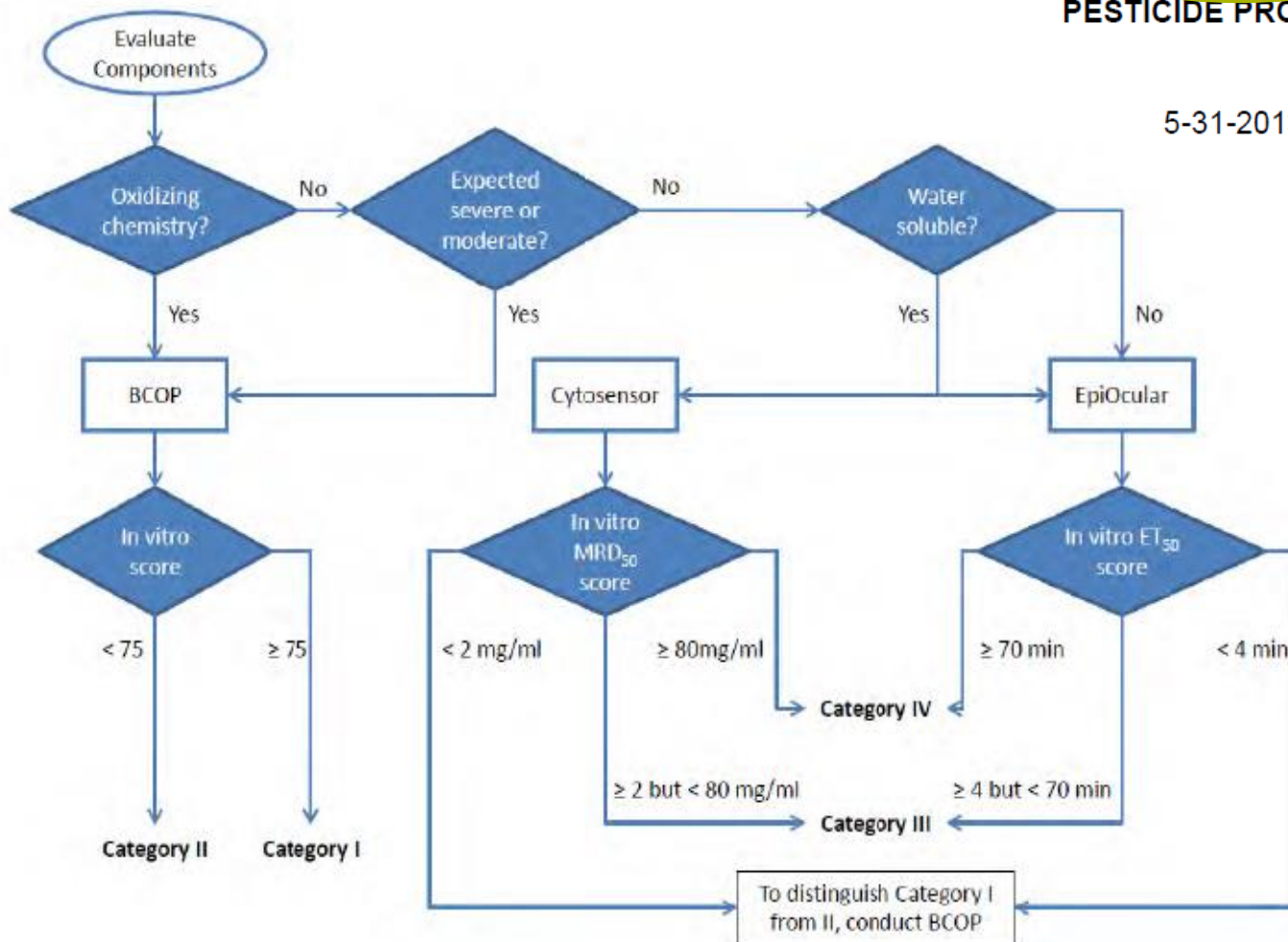
Guidance for Waiving or Bridging of Mammalian Acute Toxicity Tests for Pesticides and Pesticide Products (Acute Oral, Acute Dermal, Acute Inhalation, Primary Eye, Primary Dermal, and Dermal Sensitization)

March 1, 2012

Figure1. Decision Tree: Selection and Evaluation of Assays for Hazard Labeling

USE OF AN ALTERNATE TESTING FRAMEWORK FOR CLASSIFICATION OF **EYE IRRITATION** POTENTIAL OF EPA PESTICIDE PRODUCTS

5-31-2013

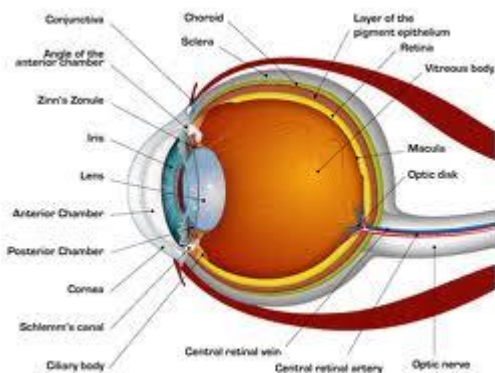
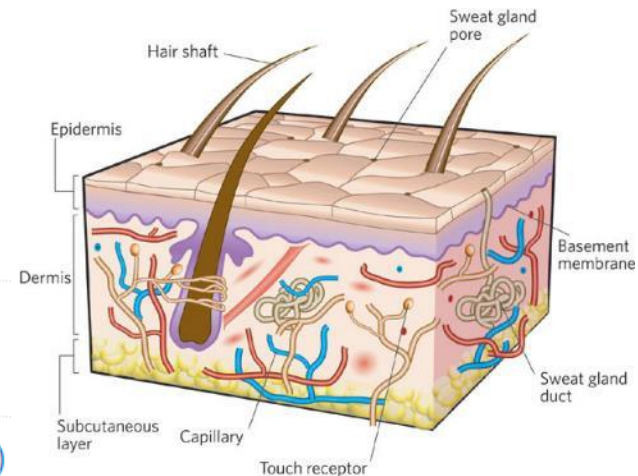


29 Jul 2016 Test No. 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method
OECD

28 Jul 2015 Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method
OECD

28 Jul 2015 Test No. 435: In Vitro Membrane Barrier Test Method for Skin Corrosion
OECD

28 Jul 2015 Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER)
OECD



27 Jun 2018 Test No. 492: Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage
OECD

27 Jun 2018 Test No. 491: Short Time Exposure In Vitro Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
OECD

27 Jun 2018 Test No. 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
OECD

09 Oct 2017 Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
OECD

09 Oct 2017 Test No. 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants
OECD

Screening
Category if corrosive/severe
Validated on single chemicals

US EPA proposal for skin sensitization

- Performance based
- Single chemical validation
- Mixed results?

Figure 2a. Schematic of the AOP “2 out of 3” defined approach. OECD TG methods for Key Events (KE) 1-3 are run in an undefined order until at least two of the three methods show consensus.

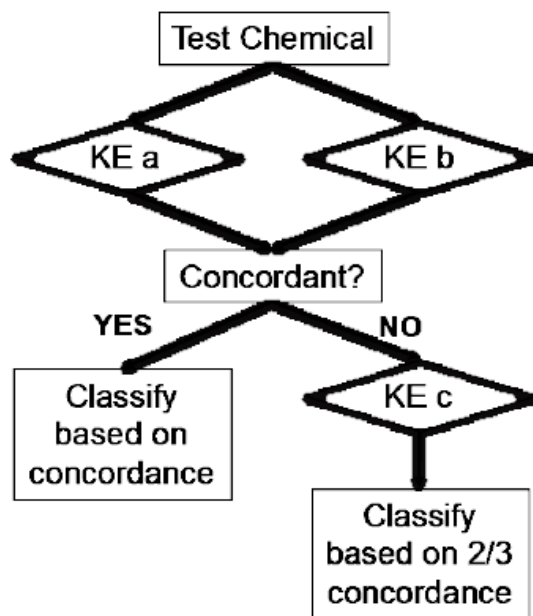
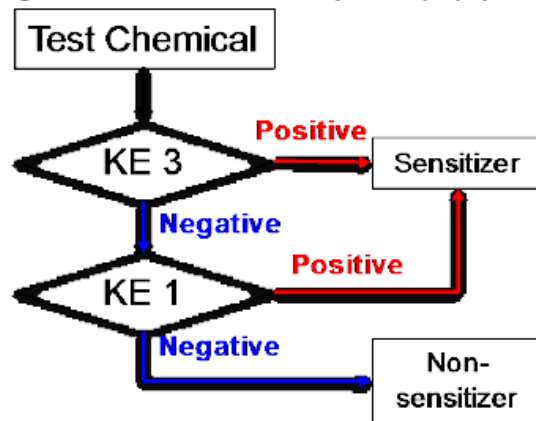


Figure 2b. Schematic of the Key Event (KE) 3/1 Sequential Testing Strategy (STS) defined approach





Beyond Acute Toxicity

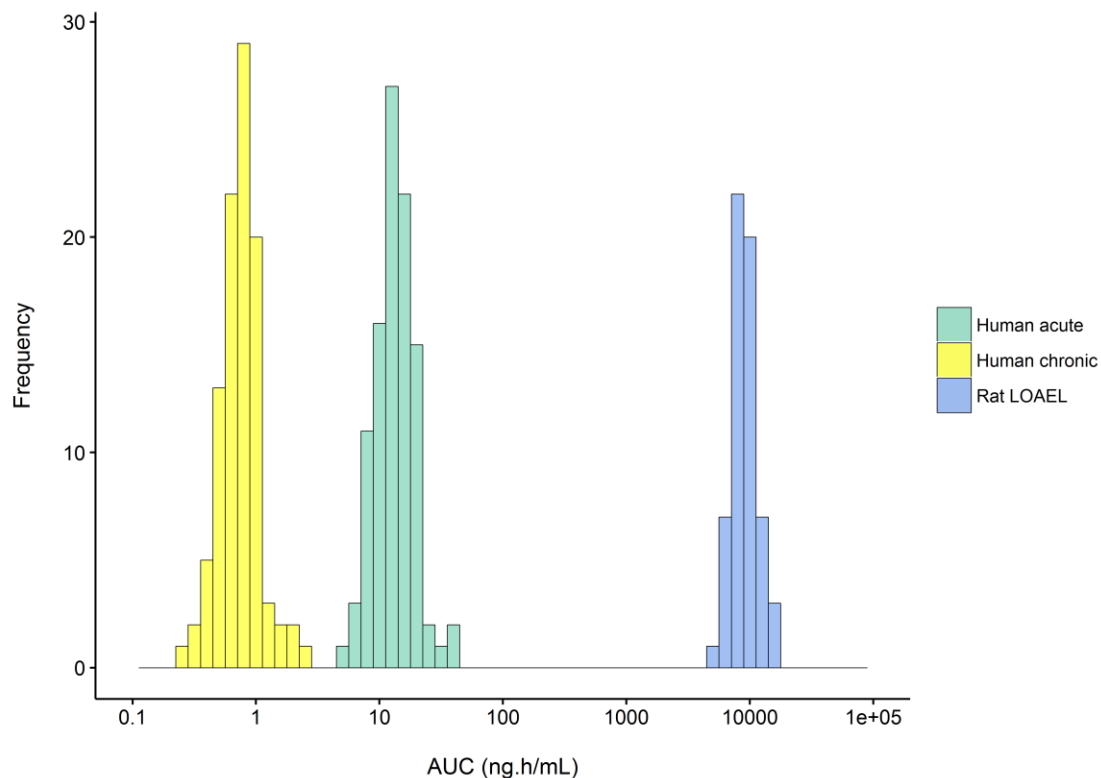
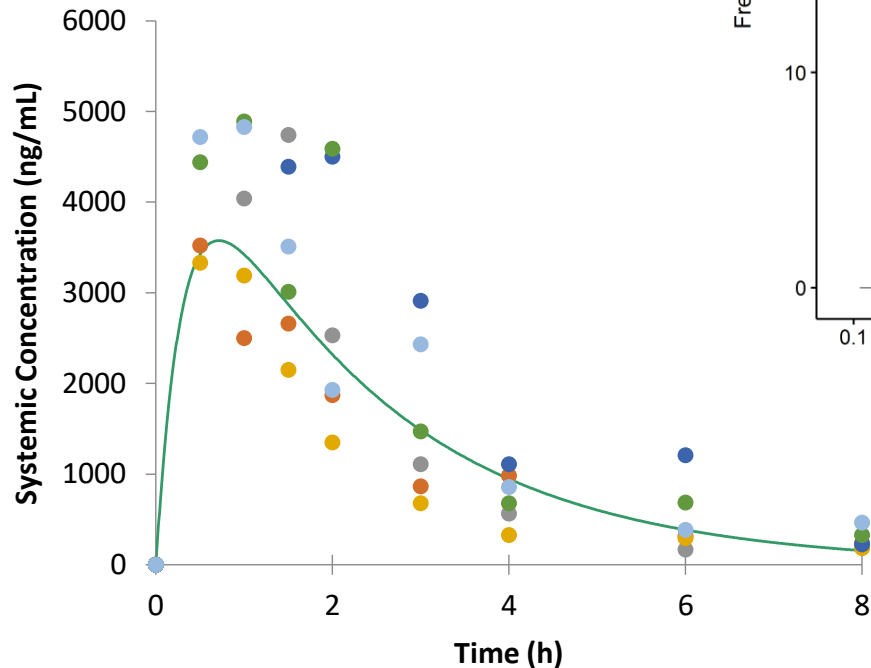
Waivers Considered by EPA's Office of Pesticide Programs (12/11-2/17)

Type of Study	Total # of Waiver Requests	Animals/study	Cost of study (USD)	Waivers Granted	Total animals saved	\$ savings (USD)	% accepted
Inhalation	288	96	350K	222	21,312	77.7M	77
Neurotoxicity	186	80	250K	163.5	13,080	40.9M	88
Dermal	57	80	310K	50	4,000	15.5M	88
Developmental	48	1700		39	66,300		81
DNT	18	880		15	13,200		83
Subchronic Dog	14	32		11	352		79
Reproductive	38	880		32	28,100		84
Immunotoxicity	223	40	70K	207	8,280	14.5M	93
Chronic/ Carcinogenicity	28	480		24	11,520		86
Subchronic Rat	12	80		10	800		83
total	912			774	166,944		85

PMRA removed 1-year dog requirement March 2016

Maximizing use of existing data

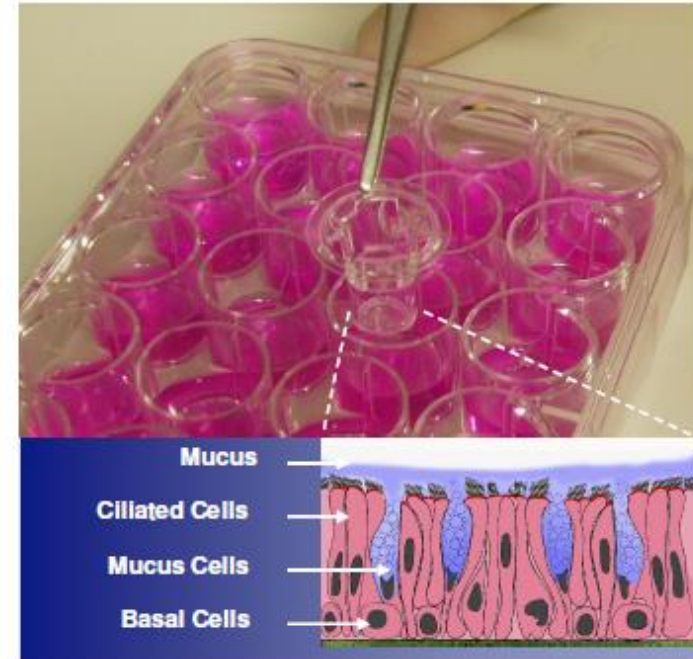
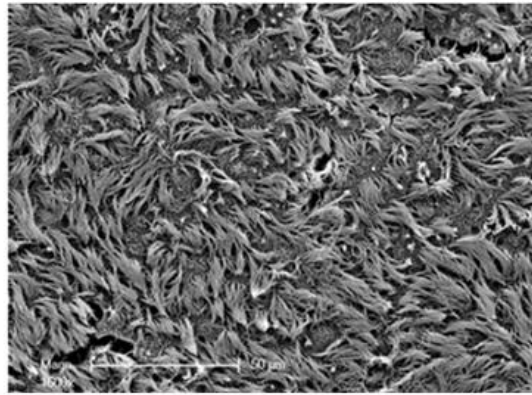
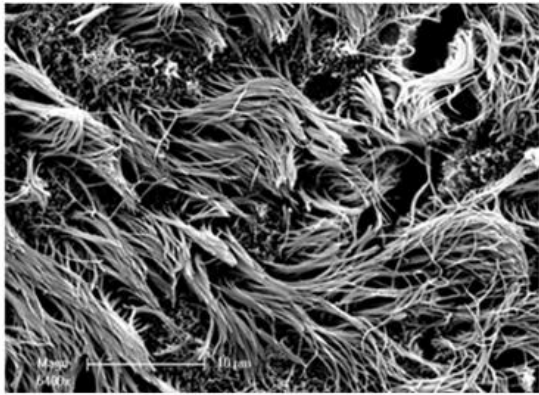
Pharmaco/toxicokinetics



Physiologically-based pharmacokinetic modelling

In vitro inhalation toxicity assessment

MucilAir™ – 3D *in vitro* cell model of human upper airway epithelium prepared from differentiated primary human cells from a single healthy donor.



Graphics from

<http://www.epithelix.com/>

Office of Pesticide Programs Update

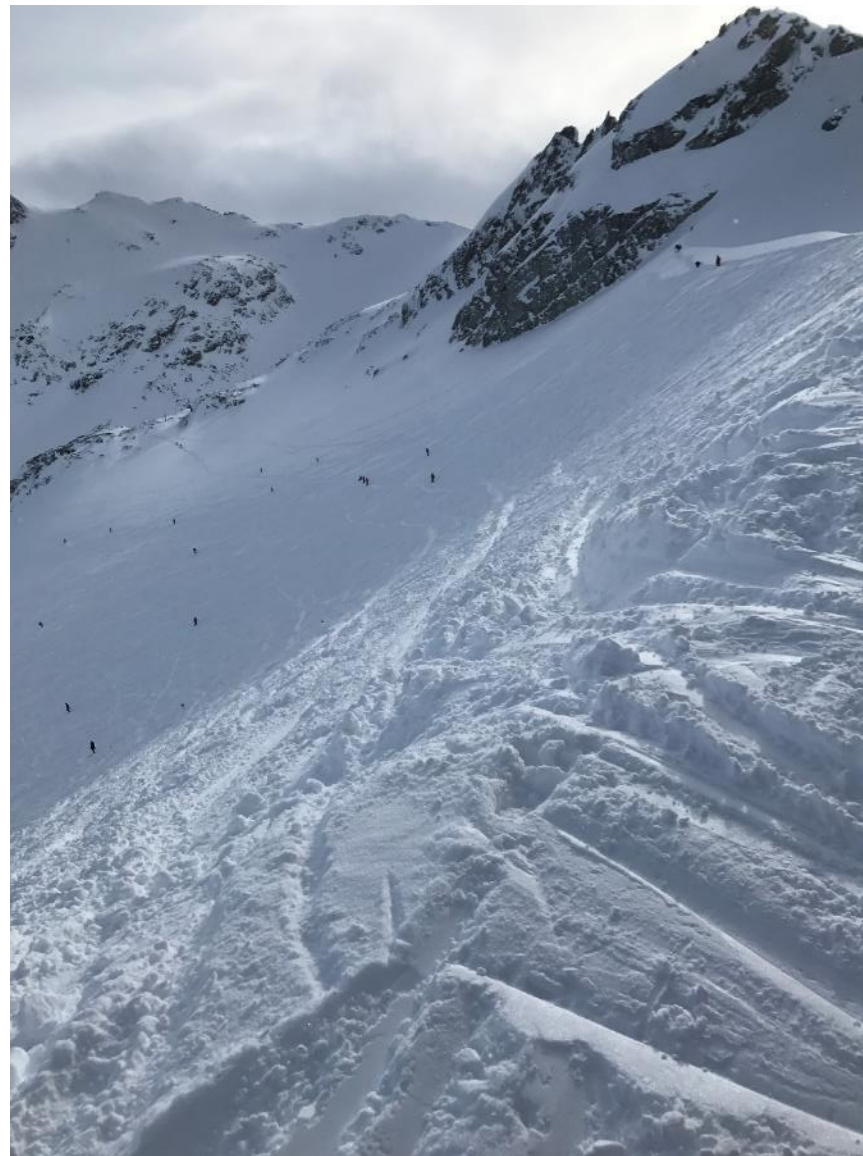


Revised: FIFRA Scientific Advisory Panel Meeting on “Evaluation of a Proposed Approach to Refine the Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM).” Request for Nominations and Notice of Public Meeting

EPA is seeking nominations for expert ad hoc members of the FIFRA Scientific Advisory Panel to evaluate a proposed approach to refine an inhalation risk assessment for point of contact toxicity. Panel members will review a case study using the pesticide chlorothalonil to evaluate a new approach methodology (NAM). The panel will meet on December 4-7, 2018.

Docket ID: EPA-HQ-OPP-2018-0517

Where are we headed



Multistakeholder *in vitro* inhalation initiative

- <https://ice.ntp.niehs.nih.gov/#!Mixtures>

National Toxicology Program
U.S. Department of Health and Human Services

Calendar & Events | News & Media

Search the NTP Website

Integrated Chemical Environment

Home | Integrator | **Formulations** | Workflows | Reference Data | About | Help

Selected Assay Categories: Acute Inhalation Toxicity

Run Search | Clear

Select Assays

- Acute Dermal Toxicity
- Acute Oral Toxicity
- Acute Inhalation Toxicity
- Primary Skin Irritation
- Primary Eye Irritation
- Dermal Sensitization

Select Reference Lists.

Enter one CASRN per line.

Query Integrator

Number of formulations = 469 (Number of active ingredients = 764 (unique: 249)). Showing 3 Endpoints.

Formulation ID	Active Ingredient	CASRN	Percent AI	Acute Inhalation Toxicity curatedEPAToxCat	Acut
▶ Abamectin B1b		ICE_4677373915		NA	NA
▶ Abamectin(92.8%)		ICE_1791765921	1		1
▶ Absolute 500 SC Fungicide		ICE_41066972582	3		3
▶ Acephate		ICE_42079364361	4		4
▶ Acephate 90 DF Insecticide		ICE_2113214488	4		4
▶ Acequinocyl		ICE_588976947	3		3
▶ Acetochlor		ICE_41652406964	4		4
▶ Acrobat 50WP Fungicide		ICE_1963069246	4		4
▶ Acticide CBM		ICE_4615347920	3		3
▶ Acticide LA 2605-F		ICE_41842760555	2		2
▶ Acticide MKW1		ICE_41895609418	4		4
▶ Acticide PM Industrial Mildewicide		ICE_1709332886	2		2
▶ Adage Premier		ICE_870154992	4		4
▶ Advantage Plus 20 for Dogs		ICE_412455843	4		4
▶ Albaugh Glyphosphate Acid		ICE_1757011315	4		4
▶ Albaugh Trifluralin 10G		ICE_41122997002	4		4
▶ Allectus SC Insecticide		ICE_41093789115	4		4
▶ Aloft LC G Insecticide		ICE_4337369718	4		4
▶ Aloft LC SC Insecticide		ICE_1577187151	3		3
▶ Ametryn		ICE_4889033908	4		5
▶ Amicarbazone Herbicide		ICE_41835701855	4		4
▶ Aminopyralid		ICE_998364049	4		5
▶ Amitraz(98%)		ICE_4791572937	4		4
▶ Amore DF		ICE_4879871432	4		5
▶ Amtide Tebuconazole 45 WDG Fungicide		ICE_1253614018	4		4
▶ Aproach Fungicide		ICE_42109571986	4		5

2016 workshop co-organized by the PETA International
Science Consortium and NICEATM

WORKING GROUP 1
Establish a
database of existing
acute inhalation
toxicity data

WORKING GROUP 2
Optimize (Q)SAR
models

WORKING GROUP 3
Prepare a review on
mechanisms of acute
inhalation toxicity,
dosimetry
considerations, &
available non-animal
methods

WORKING GROUP 4
Design a non-animal testing approach and conduct a
proof-of-concept study

Clippinger, Allen, et al. *Toxicol In Vitro*. 2018;52:131-145

Clippinger, Allen, et al. *Toxicol In Vitro*. 2018;48:53-70

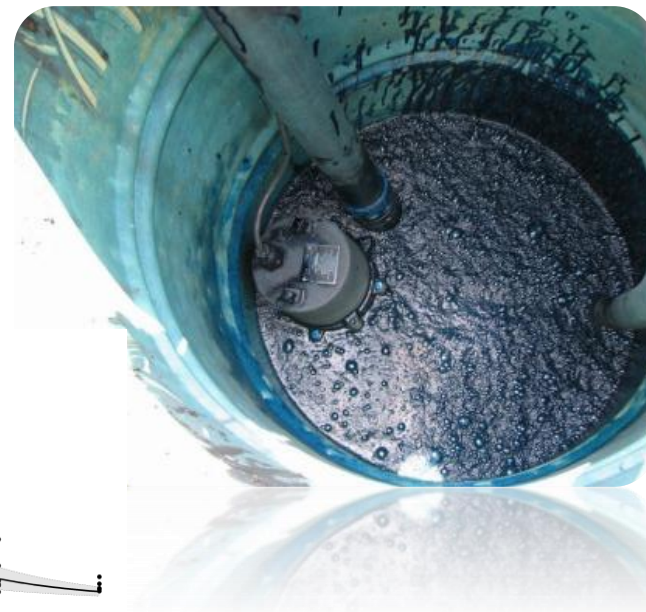
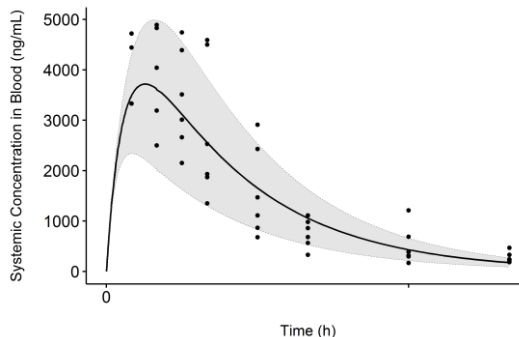
www.piscltd.org.uk/inhalation

Integrated Chemical Environment: <https://ice.ntp.niehs.nih.gov/>

Utilize existing data

- Complex mixtures
- Modelling
- Bridging:
 - Retrospective
 - Establish equivalence
 - Consistent submission & review

What we need



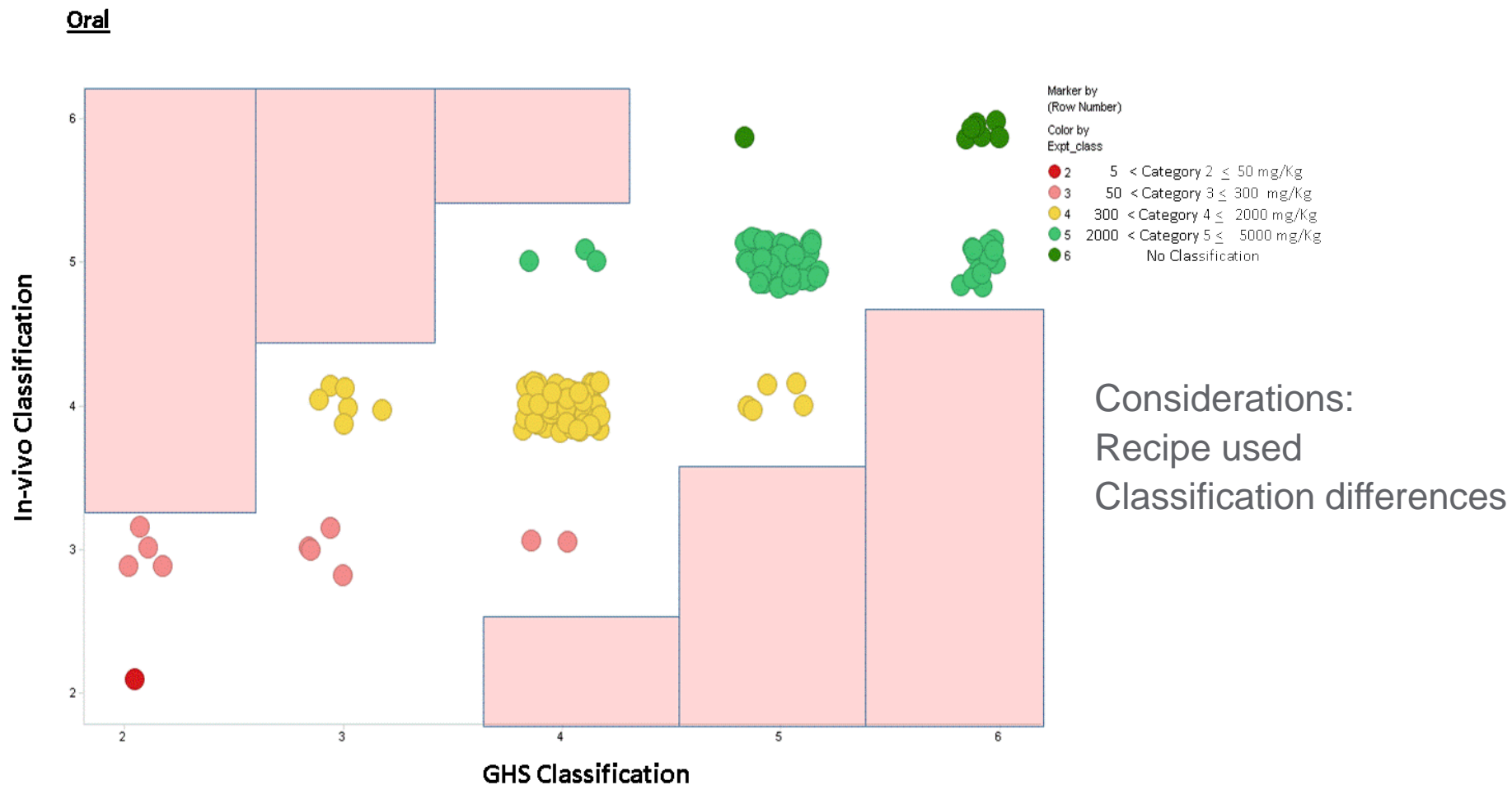
Process to assess existing data

PROCESS FOR EVALUATING & IMPLEMENTING ALTERNATIVE APPROACHES TO TRADITIONAL *IN VIVO* ACUTE TOXICITY STUDIES FOR FIFRA REGULATORY USE 2/4/2016

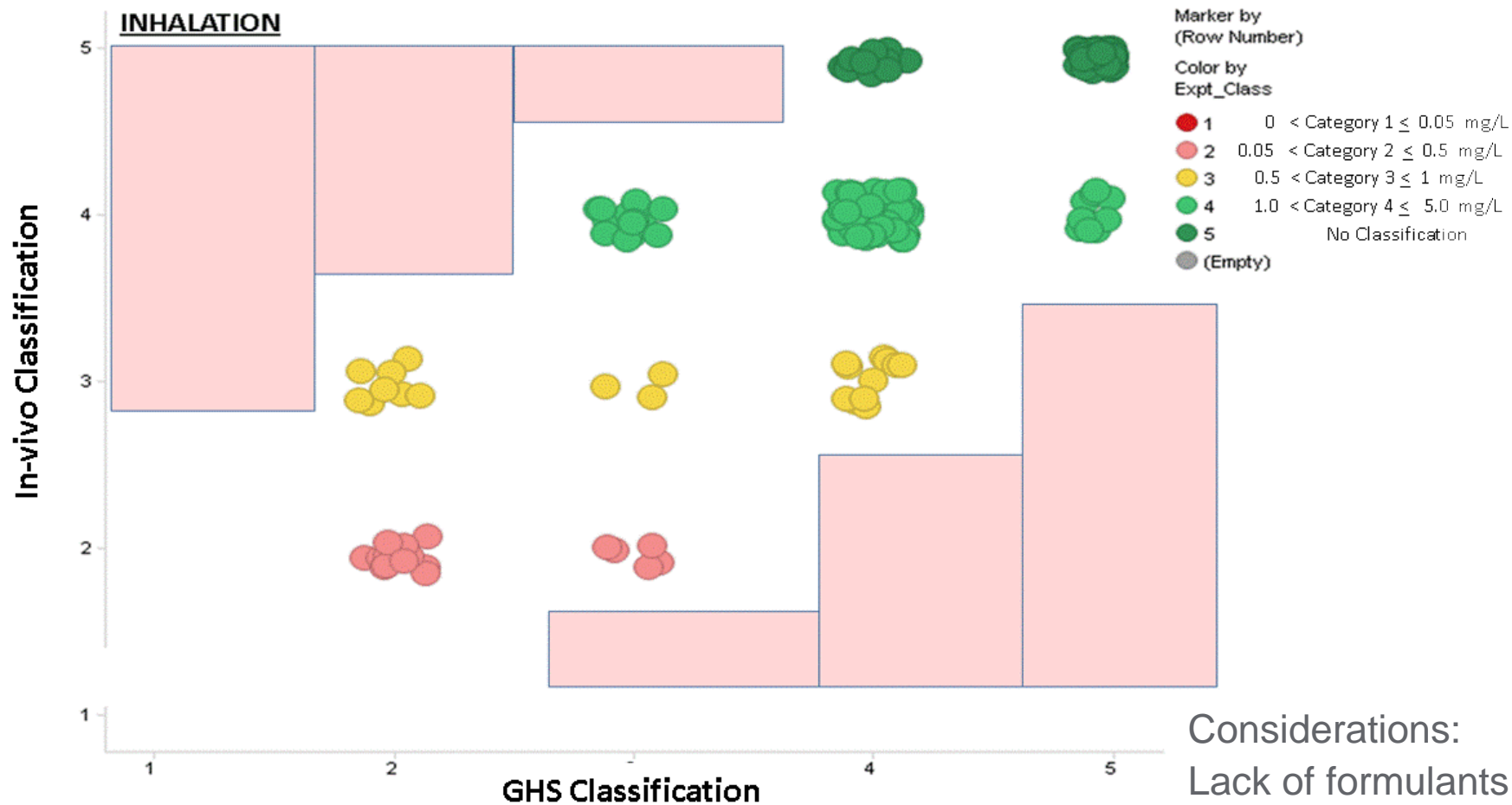


Once a proposed alternative method(s) is accepted as a suitable candidate, the evaluation process would consist of a number of steps. First, existing data generated using the alternative method(s) will be collated and organized (for example in a spreadsheet or database). The data could be previously generated or generated explicitly for the purpose of informing an evaluation of regulatory applicability. This data compilation could be accomplished in various ways. For example, a coalition of interested companies could work with a **neutral** party to collect and aggregate data so that aggregate results could be reported without disclosure of confidential business information (CBI), or data could be compiled from the open scientific literature.

Acute toxicity estimate (ATE): Acute oral



ATE: Acute inhalation



Considerations:
 Lack of formulants data
 Physical form e.g. milling

Multistakeholder *in vitro* eye irritation initiative

- 29 active ingredients, 232 formulations, 6 intermediates

Current *in vivo* - *in vitro* paired data

BCOP	Epiocular	ICE	NRR	CAMVA
132	172	91	68	4

- No clear outcome
- Prospective study
- Exploring *in silico*



Multistakeholder *in vitro* skin irritation initiative

- Accurate categorization
- Revised OECD TG 439
- AMCP & agricultural products
 - Comparison new *in vitro* data with retrospective *in vivo* results

Regulator	Categories				
EPA	IV Non to Mild	III Moderate	II Severe	I Corrosive	
PMRA	Non to Slightly	Mildly	Moderately	Severely	Corrosive
GHS	Not classified	Mild	Irritating	Corrosive (1, 1A,1B,1C)	

In vitro dermal absorption

- Part of a “triple pack”
- Retrospective
 - PMRA: fate of skin bound residue
 - EPA: decision comparison



Where we need help



Data needs



TABLE 1

URINARY EXCRETION AFTER INTRAVENOUS ADMINISTRATION*

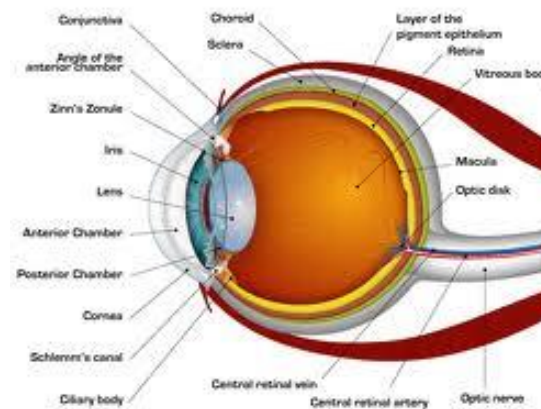
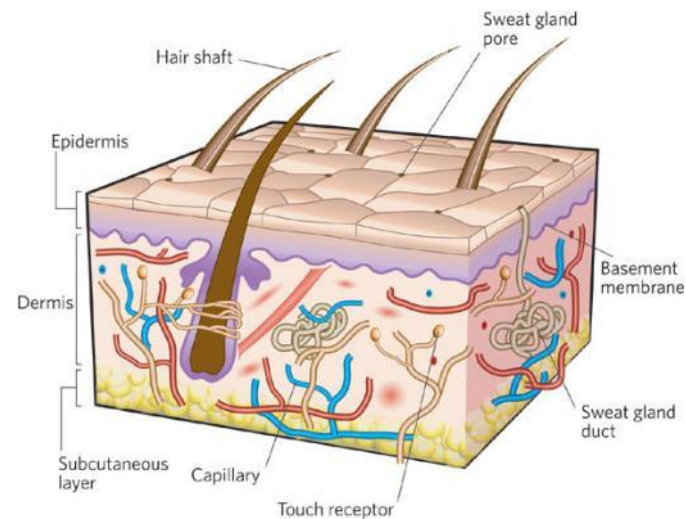
Compound	Excretion rate (% dose/hr) (time period in hr)								Total excretion		Half-life (hr)
	0-4	4-8	8-12	12-24	24-48	48-72	72-96	96-120	% Dose	SD	
Azodrin	1.816	2.721	1.701	1.000	0.679	0.341	0.173	0.088	67.7	5.3	20
Ethion	0.832	1.041	1.892	0.791	0.316	0.123	0.071	0.065	38.4	3.6	14
Guthion	1.513	1.204	1.590	1.041	0.813	0.458	0.257	0.127	69.5	6.9	30
Malathion	12.949	5.571	2.420	0.368	0.052	0.017	0.008	0.004	90.2	9.7	3
Parathion	0.035	1.321	2.508	1.124	0.469	0.135	0.059	0.037	45.8	5.3	8
Baygon	10.361	7.290	1.478	0.192	0.064	0.053	0.047	0.043	83.8	7.2	8
Carbaryl	0.459	0.394	0.211	0.102	0.037	0.021	0.011	0.008	7.4	2.2	9
Aldrin	0.224	0.091	0.113	0.040	0.023	0.013	0.011	0.008	3.6	0.9	6
Dieldrin	0.038	0.067	0.074	0.046	0.046	0.013	0.015	0.008	3.3	1.0	28
Lindane	0.688	0.611	0.552	0.244	0.232	0.132	0.125	0.102	24.6	6.1	26
2,4-D	3.001	4.003	5.312	1.728	0.737	0.275	0.153	0.097	100.0	2.5	13
Diquat	9.328	1.544	1.825	0.292	0.127	0.059	0.054	0.045	61.2	16.0	4

PESTICIDE PENETRATION IN MAN

* Mean values of urinary recovery of ¹⁴C for 5 days after single iv administration. There were 6 subjects for each compound.

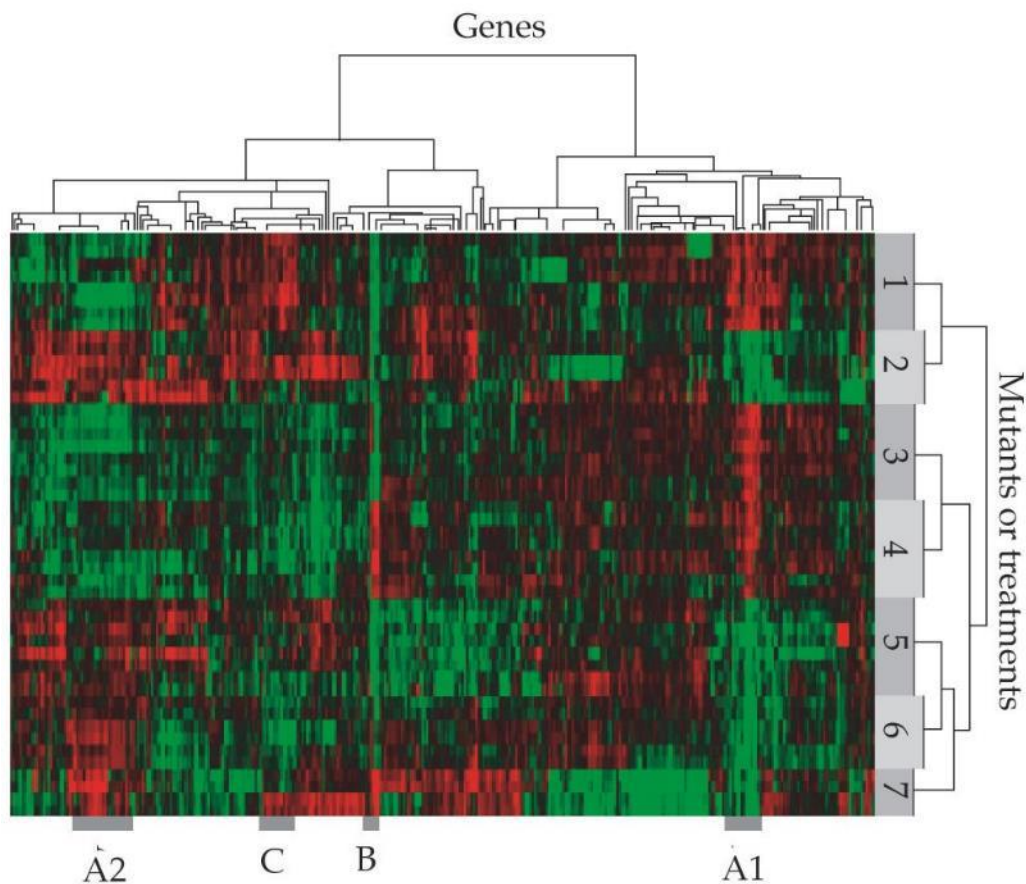
Eye & Skin Irritation

- Persistence or reversibility
 - Mimic a real eye
- Distinguish between:
 - Corrosive < Irritating < Non-Irritating
- When a negative is a negative?



Skin Sensitisation

- Complex mixture validation
- Correlation of AOP to human risk of sensitization
- Genomics approaches
 - Proprietary databases



Consistent regulation & assessment

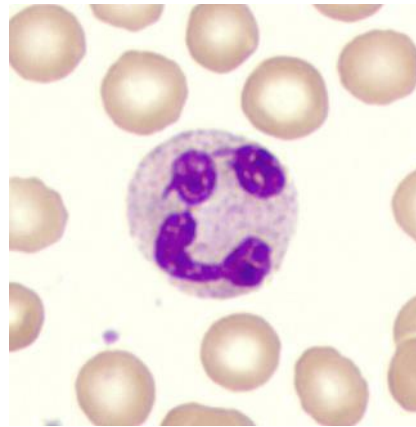
	Category 1	Category 2	Category 3	Category 4
Oral LD₅₀ mg/kg				
EPA	≤ 50	>50 to 500	> 500 to 5000	> 5000
PMRA	< 500	500 to 1000	1000 to 2000	> 2000
GHS ^{1,2}	≤ 5	5 to 50	50 to 300	300 to 2000
Inhalation LC50 mg/L				
EPA	≤ 0.05	> 0.05 to 0.5	>0.5 to 2	> 2
PMRA	< 0.05	0.05 to 0.5	0.5 to 2.0	> 2
GHS ¹	≤ 0.05	> 0.05 to 0.5	0.5 to 1	1 to 5

¹Version 5

²Category 5: 2000 to 5000 mg/kg

The reason why new concepts in any branch of science are hard to grasp is always the same; contemporary scientists try to picture the concept in terms of ideas which existed before.

Freeman Dyson 1958



Predictive
Efficient
Accepted

