Upcoming Webinar

Webinar 6: (Zebra)fish embryo acute toxicity test to predict short-term toxicity to fish (and beyond)

April 14, 2015

11am ET, 4pm GMT

Marlies Halder, EURL ECVAM

Thomas Braunbeck, University of Heidelberg

Scott Belanger, Procter & Gamble

Please feel free to contact the PETA International Science Consortium Ltd.,

for assistance in avoiding animal testing

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Alternative approaches to mammalian acute systemic toxicity testing

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Outline

- Regulatory *in vivo* tests
- EU Regulatory requirements
- Mechanistic understanding
- Efforts to develop non-animal methods
- EURL ECVAM Strategy

In vivo tests

General adverse effects resulting from **single or multiple exposure** to a substance or mixture within **maximum 24 hours** and during an **observation period of 14 days**

ORAL	DERMAL	INHALATION
OECD TG420: **Fixed Dose Procedure** (5-7 animals)	OECD TG402: **Classical LD_{50}** (10-30 animals)	OECD TG403: **Classical LC_{50}** (40-80 animals)
OECD TG423: **Acute Toxic Class** (6-7 animals)		OECD TG436: **Acute Toxic Class** (6-9 animals)
OECD TG425: **Up-and-Down Procedure** (5-9 animals)		

Endpoint: **death** or evident signs of toxicity (only TG420)

Classification and labelling criteria – EU CLP

Classification LD_{50}/LC_{50} values Oral (mg/kg b.w.) Dermal (mg/kg b.w.) Gases (ppmV) Vapours (mg/l) Dusts and Mists (mg/l)	Category 1 $LD_{50} \leq 5$ $LD_{50} \leq 50$ $LC_{50} \leq 100$ $LC_{50} \leq 0.5$ $LC_{50} \leq 0.05$	Category 2 $5 < LD_{50} \leq 50$ $50 < LD_{50} \leq 200$ $100 < LC_{50} \leq 500$ $0.5 < LC_{50} \leq 2.0$ $0.05 < LC_{50} \leq 0.5$	Category 3 $50 < LD_{50} \leq 300$ $200 < LD_{50} \leq 1000$ $500 < LC_{50} \leq 2500$ $2.0 < LC_{50} \leq 10$ $0.5 < LC_{50} \leq 1.0$	Category 4 $300 < LD_{50} \leq 2000$ $1000 < LD_{50} \leq 2000$ $2500 < LC_{50} \leq 20000$ $10 < LC_{50} \leq 20$ $1.0 < LC_{50} \leq 5$
Pictograms				
Signal Word	Danger	Danger	Danger	Warning
Hazard statement	Fatal • if swallowed • in contact with skin • if inhaled	Fatal • if swallowed • in contact with skin • if inhaled	Toxic • if swallowed • in contact with skin • if inhaled	Harmful • if swallowed • in contact with skin • if inhaled
Precautionary statements: • **Prevention** • **Response** • **Storage** • **Disposal**	ORAL: same precautionary statements for categories 1, 2, 3 DERMAL: same precautionary statements for categories 1 & 2, INHALATION: same precautionary statements for categories 1 & 2			

Supporting elements of risk assessment and risk management

- Setting of occupational exposure limits
- Chemical emergency response planning

Exposure occurs via accident or contaminated land

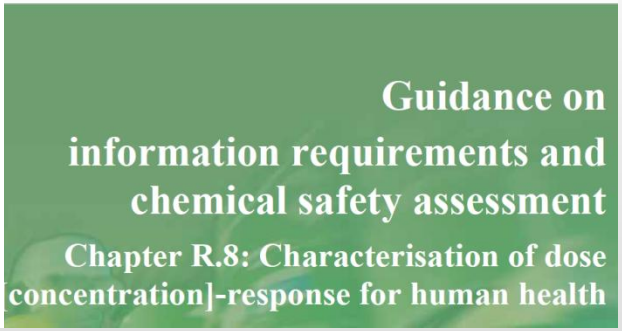
✓ REACH and acute DNELs

(**workers exposed to high peak concentrations**)

ECHA
http://echa.europa.eu

APPENDIX R. 8-8 Acute toxicity

In summary, for some substances, notably substances for which an acute toxicity hazard (leading to C&L) has been identified and for which the exposure assessment (the tentative exposure scenario) has predicted high peaks (because of, e.g., high volatility or specific use patterns), the long-term DNELs may not ensure a sufficient level of protection after peak exposure. Particular account should be taken of health effects which are not of the same type as those which drive the long-term DNEL. Still, all of the available evidence and not only the acute toxicity studies should be used to determine the most appropriate toxicological effect on which to base the derivation of the DNEL for acute toxicity. As a rule of thumb, a $DNEL_{acute}$ should be set for acutely toxic substances if actual peak exposure levels significantly exceed the long-term DNEL. For such cases, a $DNEL_{acute}$ need to be set and assessed in relation to the peak exposure levels that humans may experience.



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EU Regulatory Framework

Cosmetics Regulation
All cosmetic ingredients independent of tonnage level

Acute toxicity is part of base set requirement

CLP Regulation
All substances and mixtures supplied in EU

Four hazard categories (oral, dermal, inhalation)

REACH Regulation
All substances manufactured/imported in EU in quantities ≥ 1tpy

≥ 1tpy: oral route; ≥ 10tpy: oral and a 2nd exposure route

Acute Systemic Toxicity

Plant Protection Products Regulation

All products and active substances marketed in EU independent of tonnage

Oral route always
dermal and inhalation routes if required

Pharmaceuticals

Discontinued

Biocides Regulation

All products and active substances marketed in EU independent of tonnage

Oral and
a 2nd exposure route

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Information requirements under REACH

ANNEX VII

STANDARD INFORMATION REQUIREMENTS FOR SUBSTANCES MANUFACTURED OR IMPORTED IN QUANTITIES OF ONE TONNE OR MORE (1)

COLUMN 1 STANDARD INFORMATION REQUIRED	COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1
8.5. Acute toxicity	8.5. The study/ies do(es) not generally need to be conducted if: — the substance is classified as corrosive to the skin.
8.5.1. By oral route	The study need not be conducted if a study on acute toxicity by the inhalation route (8.5.2) is available.

Information requirements under REACH

ANNEX VIII

STANDARD INFORMATION REQUIREMENTS FOR SUBSTANCES MANUFACTURED OR IMPORTED IN QUANTITIES OF 10 TONNES OR MORE (1)

COLUMN 1 STANDARD INFORMATION REQUIRED		COLUMN 2 SPECIFIC RULES FOR ADAPTATION FROM COLUMN 1	
8.5.	Acute toxicity	8.5.	The study/ies do(es) not generally need to be conducted if: — the substance is classified as corrosive to the skin. In addition to the oral route (8.5.1), for substances other than gases, the information mentioned under 8.5.2 to 8.5.3 shall be provided for at least one other route. The choice for the second route will depend on the nature of the substance and the likely route of human exposure. If there is only one route of exposure, information for only that route need be provided.
8.5.2.	By inhalation	8.5.2.	Testing by the inhalation route is appropriate if exposure of humans via inhalation is likely taking into account the vapour pressure of the substance and/or the possibility of exposure to aerosols, particles or droplets of an inhalable size.
8.5.3.	By dermal route	8.5.3.	Testing by the dermal route is appropriate if: (1) inhalation of the substance is unlikely; and (2) skin contact in production and/or use is likely; and (3) the physicochemical and toxicological properties suggest potential for a significant rate of absorption through the skin.

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Mechanistic understanding

From: ECVAM Workshop on Strategies to Replace Acute Systemic Toxicity Testing (Gennari et al., 2004 ATLA)

Mechanisms common to many cell types responsible for cellular failure or death
ROS formation
Energy production and metabolism
Mitochondrial function
Glycolysis
Membrane structure and function
Protein turnover
Gene regulation
Cell communication: intracellular signalling, cell-cell interaction

Organ-specific functions commonly compromised in organ failure
Kidney: transport, filtration
Liver: metabolic transformation (xenobiotics, nutrients, protein regulation and excretion)
CNS: neurotransmission, structural integrity
Cardiovascular system: electrical conduction, contraction
Lung/respiratory system: Membrane integrity, gas exchange
Blood: oxygen transport, white cell production
Gastrointestinal tract: Transport (hydration)

Mechanistic understanding

From: ICCVAM-NICEATM/ECVAM, JaCVAM Workshop Report on Acute Chemical Safety Testing. NIH, 2009

Key processes associated with acute human poisoning
General cellular function
Neuronal transmission (central and peripheral)
Na^+, K^+ ATPase pump
Xenobiotic and aerobic metabolism
Cardiac conduction
Receptor activity
Immune response

Toxicokinetic factors

- ADME properties govern the toxicity of a chemical *in vivo*

- They need to be incorporated in successful testing strategies and approaches together with the assessment of basal cytotoxicity, cell-specific toxicity and cell-specific function

Draft EURL ECVAM Strategy Report on Toxicokinetics

Efforts to develop non-animal methods

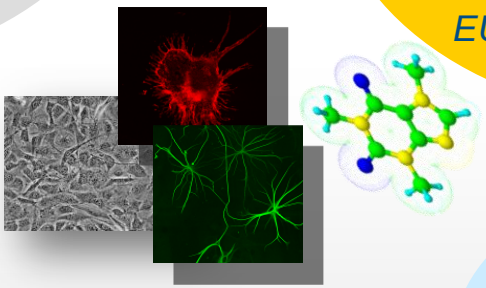
MEIC programme (*1989-1996*)

Registry of Cytotoxicity (*Halle, 2003*)

Basal cytotoxicity is an important event in acute systemic toxicity

NICEATM/ECVAM Validation of NRU cytotoxicity assays
NIH, 2006; OECD GD129 (2010)
Schrage et al., 2011

EURL ECVAM Validation of 3T3 NRU
EURL-ECVAM REC (2013)



Combination of cytotoxicity, toxicokinetic parameters, and organ-specific toxicity

….and others…

Use of QSAR models

Norlén et al, 2010 EUR 24639 EN
Norlén et al, 2014 ATLA

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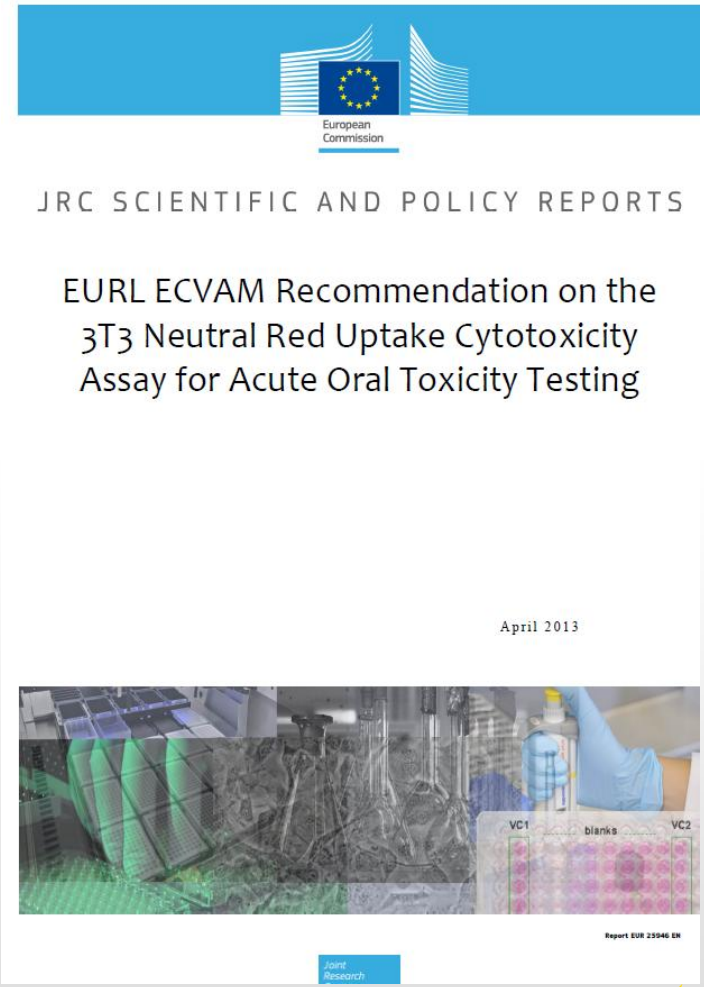
3T3 NRU Recommendation



- Valuable component of a WoE or ITS approach for supporting identification of non-classified substances (threshold 2000 mg/kg).
- Assessment of other data sources is needed.
- Particularly relevant for industrial chemicals.
- Care must be taken in interpreting results when chemicals need metabolic activation or are detoxified.
- Positive results (classified substances) cannot be used as reliable indication of acute oral toxicity.

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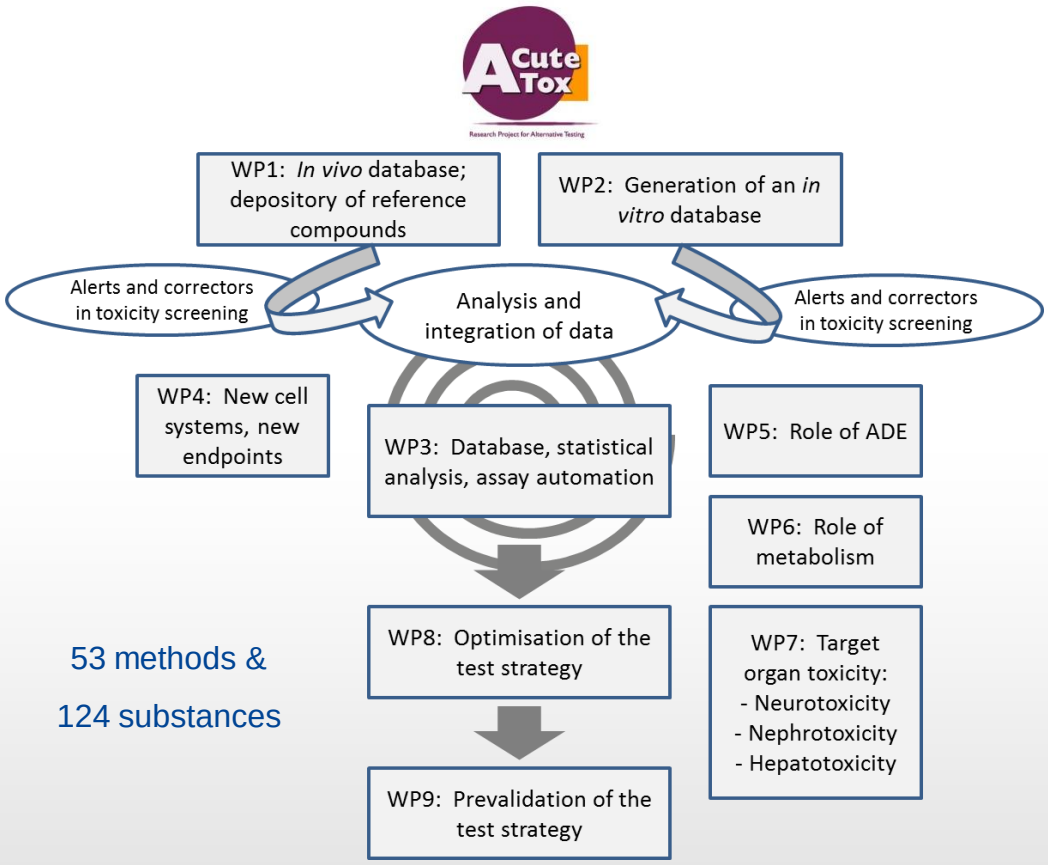
Non-animal testing strategy



53 methods &

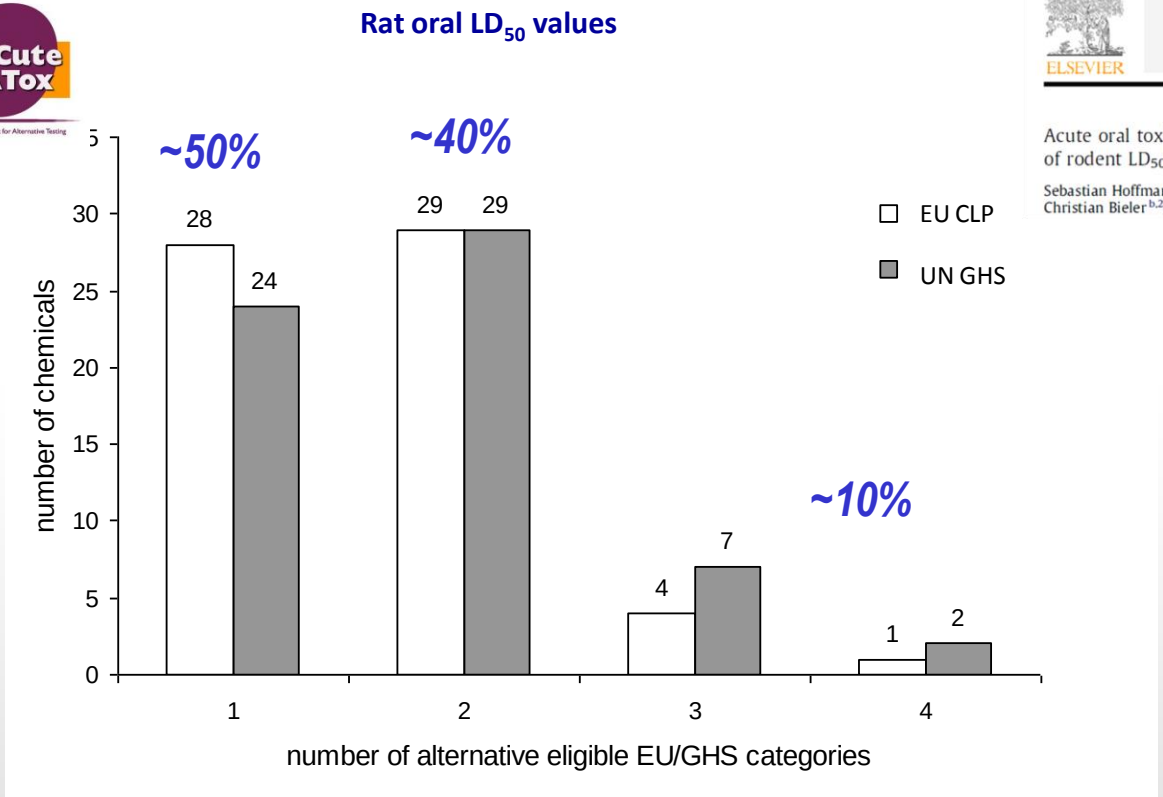
124 substances

Toxicology in Vitro, 2013, Special Issue 27(4): 1347 - 1424

Overall outcome

- Supports the use of 3T3 NRU within a testing strategy to identify non-classified chemicals.
- Classification of compounds in EU CLP toxicity cat. 1-4 did not improve significantly with the proposed strategies.
- Proposed strategies have a tendency to over-predict toxicity (kinetic parameters need further evaluation)
- Target organ specific *in vitro* assays alert for specific toxicity (e.g. neurotoxicity) used in combination with 3T3 NRU assay.

Evaluation of animal data – consistency of classification



Regulatory Toxicology and Pharmacology 58 (2010) 395–407

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

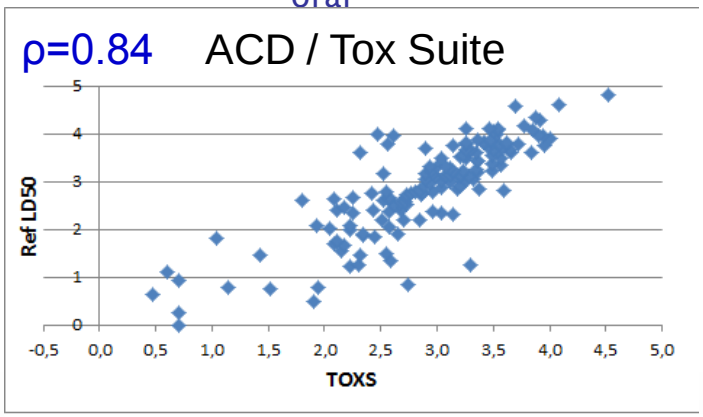
Acute oral toxicity: Variability, reliability, relevance and interspecies comparison of rodent LD_{50} data from literature surveyed for the ACuteTox project

Sebastian Hoffmann [a,1], Agnieszka Kinsner-Ovaskainen [a], Pilar Prieto [a,*], Inge Mangelsdorf [b], Christian Bieler [b,2], Thomas Cole [a]

With at least 90% probability ~50% of the substances would fall into only one toxicity category and ~40% would fall within two neighbouring categories

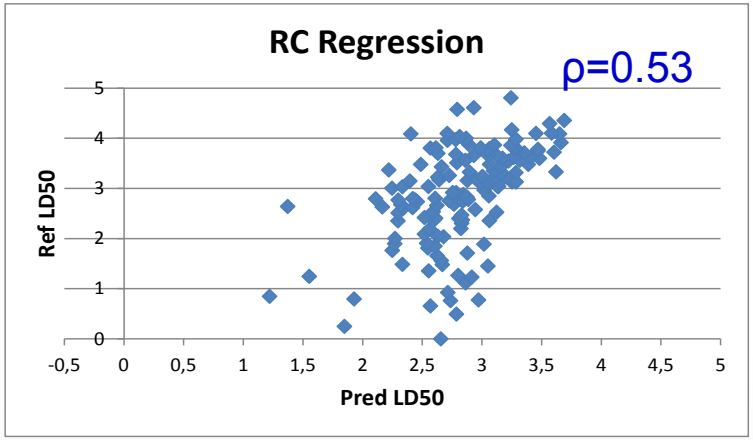
Combination of QSARs models and cytotoxicity

Software for acute oral toxicity (LD_{50}) prediction oral



Norlén et al, 2012. JRC Report. EUR 25473 EN.

3T3 NRU cytotoxicity assay & regression model for LD_{50} prediction



Prediction of three toxicity classes

- The ACD/Tox Suite and U.S. EPA/ T.E.S.T. best performing when used individually.
- The 3T3 NRU showed the lowest accuracy.
- The use of the five methods in combination did not result in significantly better predictive performance.

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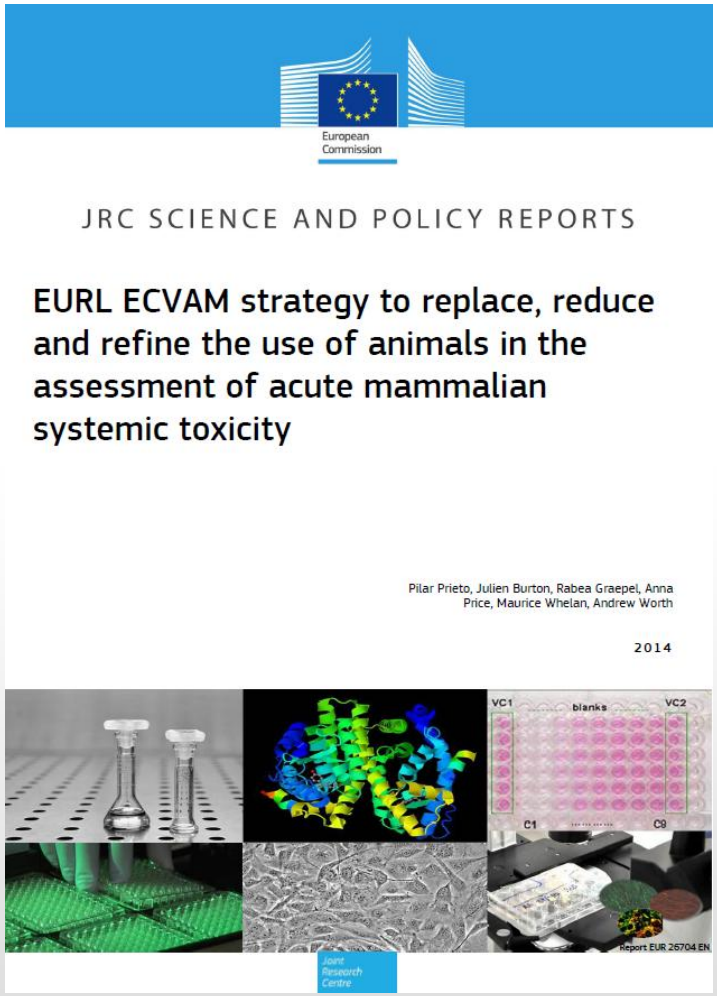
EURL ECVAM strategy



- **Specify aims and associated objectives to progress the field of acute systemic toxicity that reduce and eventually replace animal use.**
- **Provide a framework for the prioritisation of alternative methods submitted to EURL ECVAM for validation.**

Ultimate aim: to propose solutions that can satisfy regulatory requirements under several pieces of EU legislation

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Aims and Objectives

OBJECTIVES

Strategic Aim: *Reduction & Replacement*	Strategic Aim *Refinement*
Development and/or optimisation of mechanistically relevant alternative methods as elements for use within Integrated Approaches to Testing and Assessment (IATA)	Support efforts to avoid the use of lethality as endpoint for acute systemic toxicity testing
Explore the use of existing repeated dose data to derive classification and labelling for acute oral toxicity	Support the revision of current *in vivo* acute dermal toxicity test
Route-to-route, in vitro-to-in vivo & inter-species extrapolation	
Support the development of scientifically based waiving arguments to avoid animal testing in acute systemic toxicity studies	*https://eurl-ecvam.jrc.ec.europa.eu/eurl-ecvam-strategy-papers/strategy-acute-mammalian-systemic-toxicity*

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Strategic Aim Reduction & Replacement

Objective: Development and/or optimisation of mechanistically relevant alternative methods for use within IATA

- Information provided by relevant *in vitro* assay, including cytotoxicity assays, is expected to contribute to the WoE in future IATA for this endpoint.
- EURL ECVAM proposes to explore options to make better use of alternative methods e.g. validated 3T3 NRU test method.

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Strategic Aim Reduction & Replacement

Objective: Explore the use of repeated dose studies to support C&L for acute oral systemic toxicity

- Build on the work by *Bulgheroni et al (2009) Reg.Tox.Pharm*: Possibility to identify non-classified substances from 28-day NOAEL values.

  The threshold **NOAEL > 200 mg/kg b.w**. allowed the correct identification of 63% of nontoxic compounds, while less than 1% of harmful compounds were misclassified as nontoxic

- EURL ECVAM Survey "Acute Systemic Toxicity Testing - Exploring Waiving Opportunities“

- Relevant for the **REACH 2018 deadline**, at 10-100 tpy, for which repeat dose 28 day data are required (**Annex VIII**)

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Strategic Aim Reduction & Replacement

Objective: Development of scientifically based waiving arguments to avoid animal testing in acute systemic toxicity studies

- EPAA and HSI proposals to modify REACH standard requirements for acute toxicity (Annex VIII, point 8.5)

  - ✓ Acute toxicity by routes other than oral only when indicated
  - ✓ Quantitative criteria for testing via inhalation route
  - ✓ Inhalation – ATC as preferred method (OECD TG436)
  - ✓ Quantitative criteria for testing via the dermal route
    - To allow waiving the dermal toxicity test if not classified by the oral route (i.e. oral LD_{50} > 2000 mg/kg) **agreed at CARACAL 15** (July 2014)

- OECD WNT approved project: to develop guidance for waiving mammalian acute toxicity tests, including acute systemic toxicity testing, for pesticides and biocides

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Strategic Aim Refinement

Objective: Continue efforts to avoid the use of lethality as endpoint for acute systemic toxicity

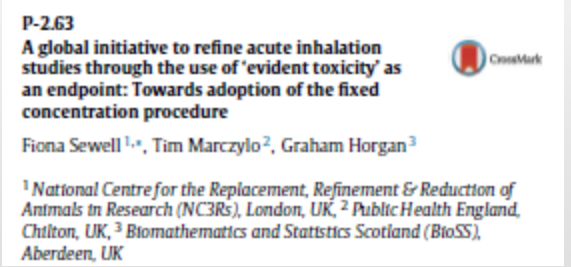
Substitution of lethality by more humane clinical signs is encouraged by Directive 2010/63/EU and OECD GD 19

Two ongoing activities supported by EURL ECVAM:

- EPAA project – integration of evident toxicity as endpoint.
- NC3Rs activities to support acceptance at OECD level of FCP for acute inhalation (OECD TG 433).

Preliminary results: signs such as body weight loss, irregular respiration, gasping, ano-genital staining or hypoactivity are highly predictive (positive predictive value >90%) of severe toxicity or death at the next highest dose.

(Sewell et al, 2014 . Abstracts / Toxicology Letters 229S (2014) S40–S252)



P-2.63

A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an endpoint: Towards adoption of the fixed concentration procedure

Fiona Sewell [1,*], Tim Marczylo [2], Graham Horgan [3]

[1] National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs), London, UK, [2] Public Health England, Chilton, UK, [3] Biomathematics and Statistics Scotland (BioSS), Aberdeen, UK

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Strategic Aim Refinement

Objective: Support the revision of current *in vivo* acute dermal toxicity studies

OECD WNT approved project to either revise or replace the OECD TG 402 (acute dermal toxicity testing) in line with the 3Rs principles.

- ✓ Uses lethality as the primary endpoint
- ✓ Requires an average number of animals between 10 (limit test) and 30 (5 animals per sex)

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Summary

- Currently only data derived from animal tests are accepted by regulatory bodies. However, *in vivo* acute systemic toxicity studies are prohibited in EU for cosmetic substances and products. Furthermore, under REACH test on animals must only be conducted as a last resort and ANNEX XI describes how standard data requirements can be adapted.
- Efforts should be directed towards the reduction and replacement of animal tests for the identification and classification of acute systemic toxicity.
- An OECD guidance document on the use of *in vitro* cytotoxicity assays as additional tests that can be used for estimating the initial doses for test *in vivo*, exists.
- The evidence also indicates that the 3T3 NRU basal cytotoxicity assay can be used to support the identification of negatives (non-classified substances according to EU CLP), with the caveat that due to the limitations of this test method, results should always be used in combination with other information sources to build confidence in the decision not to classify a substance for acute oral toxicity.
- Information provided by mechanistically relevant *in vitro* assays as well as existing information on repeated dose toxicity is expected to contribute to the WoE in future Integrated Approaches to Testing and Assessment of this endpoint.
- Efforts should also continuo in the refinement of *in vivo* studies and in particular to avoid lethality as endpoint for acute systemic toxicity testing

3M Corporate Medical Department

- Toxicology and Strategic Services group
  - Supports all 3M businesses for human health hazard/risk assessments and toxicity testing
  - Includes all toxicologists supporting 3M Business units, and Strategic Services, which includes computational toxicology and the onsite toxicology laboratory

Acute Toxicity Needs

- 3M product line is vast and highly variable, so business support needs differ greatly

- Highly variable needs for acute toxicity data
  - GHS classification and hazard communication
  - New chemical registrations
  - Regulatory submittals
  - Risk/safety assessments
  - Raw material selection/screening

Alternative Strategies

- Goal is to avoid classic animal based acute toxicity testing (rat oral LD50, etc)
- May not be possible to avoid in some instances
- Computational and laboratory methods

Computational Approaches

- General approach at 3M is to utilize three tiered strategy for computational estimates
  - (Q)SAR
  - Structural alerts
  - Read-across

- Confidence in estimate is increased when there is consistency among results

Computational Approaches

- For acute toxicity, goal is generally GHS acute toxicity classification ranges
- Use a combination of free and subscription software and also an internal databases built to store previous estimates to add consistency to the process
- Working to build models using 3M historical test data

Computational Approaches

- Challenges for acute toxicity estimations
  - Many chemistries not well represented in commercial (Q)SAR programs
  - Lack of structural alerts for acute toxicity
  - Inconsistent results are common between approaches

Laboratory Methods

- OECD No. 129 - Guidance Document on Using Cytotoxicty Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests

- Significant challenges with solubility and reactivity with standard cell culture methods

- Investing in cell culture equipment to examine 3D respiratory tissues as possible screening tools for inhalation toxicity

Summary

- 3M is proactive at investing in alternative models for acute toxicity estimation
- Limits around capabilities of computational and laboratory methods
  - Regulatory acceptance
- Approaches to date have been useful and efforts will continue

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