



The European Partnership  
for Alternative Approaches to Animal Testing

## **EPAA Workshop: Acute Toxicity Testing Across Sectors – 16 September 2010**

**Silken Berlaymont Hotel, Brussels**

### **Contents**

1. The report and its aim .....	1
2. Executive summary and key messages .....	2
3. Setting the Scene (summary of presentations) .....	3
4. Drivers for testing .....	5
5. Redundancy of multi route testing .....	6
6. Recommended Next steps.....	8
Appendix 1: List of participants	
Appendix 2: Programme	

### **1. The report and its aim**

On 16 September 2010 the European Partnership for Alternative Approaches to Animal Testing (EPAA), a joint initiative between the European Commission and individual companies and trade federations from a variety of industry sectors, organised a workshop in Brussels to consider the conclusions of a review paper produced by its Acute Toxicity Task Force. The aim: to establish, where possible, areas of consensus among the different stakeholders about approaches to replace, reduce or refine the use of animals in acute toxicity testing.

This report seeks to highlight the main discussion points, and in particular the consensus conclusions to emerge from the workshop. The report was written by science journalist Peter Wrobel and finalised with input from the EPAA Acute Toxicity Task Force. For further details, please consult the presentations from the workshop online at [epaa.eu.com](http://epaa.eu.com).

## 2. Executive summary and key messages

The term “acute toxicity” is used to describe the adverse effects of a substance that may result from a single exposure or multiple exposures within a 24-hour period. Many different types of acute toxicity studies exist; however, for the purposes of this workshop, the focus was placed on systemic toxicity following exposure via the skin, oral ingestion or inhalation. For decades, such testing – normally to the point of lethality – has been considered indispensable to the regulatory process of establishing the relative toxicity of a substance – be it a new chemical, drug, or agrochemical product.

The requirements for acute toxicity testing in animals have been successfully challenged in some areas. In 2009, international pharmaceutical guidelines were revised to remove the requirement for stand-alone acute toxicity studies, by which time many companies had already discontinued such testing. But what about the other industry sectors that still carry out such testing, and indeed are required to do so by regulators e.g. industrial chemicals and agrochemicals?

The question is by no means ‘academic’: acute toxicity testing accounts for a significant number of tests carried out on animals today, raising large ethical and animal welfare issues in addition to the scientific concerns about how useful they are.

Spurred by progress in pharmaceutical sector, the EPAA assembled a Task Force to review the scientific basis of acute toxicity testing more widely. Its results were published in May 2010 in the journal *Toxicological Sciences*<sup>1</sup>, and the aim of the workshop was simple: to review the authors’ conclusions with a wider group of stakeholders and establish areas of consensus around them.

Those conclusions covered recommendations for moving away from study designs that involve death as an endpoint and for reducing where possible the application of stand-alone acute toxicity studies generally, including actions by regulators and policymakers worldwide. There were also specific recommendations to discontinue acute testing by the dermal route and to reduce the maximum dose to be administered.

Workshop participants are listed in *Appendix 1*, and the programme is presented in *Appendix 2*.

The workshop ended with a final feedback and conclusions session that revealed several areas of broad consensus among a stakeholder group that included the regulatory and regulated communities, academics, and public interest stakeholders. If implemented, these proposals could markedly reduce and refine the use of animals in testing while maintaining the protection of human health. The areas of consensus are:

---

<sup>1</sup> Seidle *et al.* (2010). Cross-Sector Review of Drivers and Available 3Rs Approaches for Acute Systemic Toxicity Testing. *Toxicological Sciences* 116(2), 382-96.

- The scientific drivers for acute lethality testing are very weak.
- EPAA survey. The most commonly used study type was OECD TG 423 (the Acute Toxic Class method). It was agreed that TG 420 (the Fixed Dose Procedure) is more humane and has the potential to provide more information on clinical signs observed at doses associated with toxicity. Active efforts should be made to promote acceptance of TG 420 globally.
- Limit dose. The EU and OECD discourage testing above 2g/kg bodyweight, but some countries require testing up to 5g/kg. In the pharmaceutical sector, limit doses for general toxicology have been reduced to 1g/kg. It was suggested that efforts could be made to challenge the use of doses up to 5g/kg. Questions were raised about how often systemic exposure increases when animals are administered doses above 1g/kg. One activity to consider would be collecting data on systemic exposure above 1g/kg.
- It could be beneficial to organise follow-up workshops to address the following hypothetical situation in the agrochemical and chemicals sectors: How could classification and labelling decisions be made if stand-alone acute toxicity testing were prohibited? What would be the consequence for down-stream regulations? This may highlight opportunities for waiving in 'data rich' situations.
- Existing data sets for oral/dermal concordance support the proposal that there should be no routine requirement for acute testing by the dermal route, either for individual/active substances or well-defined formulations for which the acute toxicity of individual constituents is known. Can this be incorporated into revised data requirements for agrochemical pesticides and biocides, as well as chemicals under REACH? Could relevant sectoral guidance documents be updated?
- Existing data sets do not support the same approach for inhalation testing. However, the group felt that it would be necessary to avoid the situation under REACH whereby inhalation becomes the default second route for acute testing for substances in the  $\geq 10$  tpa tonnage band (by process of elimination if dermal testing is discontinued). Waiving opportunities (e.g. consideration of physicochemical properties and the likelihood of exposure by the inhalation route) should be fully explored.
- Further work relating to oral/inhalation concordance would need to address the reasons for relatively lower concordances as compared to oral/dermal analyses.

### **3. Setting the Scene (summary of presentations)**

The opening presentation stated that the objective of the workshop was to review the recommendations of the EPAA Acute Toxicity Task Force. Why target acute toxicity? The answer: although it is a widespread legal requirement, it is contentious,

yields very little scientifically useful data, and the scientific drivers for conducting acute lethality studies could be met in ways that do not entail lethal animal testing. An added impetus was that it has already been dispensed within pharmaceutical testing, after extensive review and discussion.

Moving to the scientific and regulatory reasons for acute toxicity testing, the scope of the EPAA task force review was acute systemic toxicity testing in mammals via various routes: e.g. oral, skin and inhalation.

The review looked at the OECD test guidelines as well as regulations/data requirements for acute toxicity testing across different world regions and industrial sectors, and supplemented this with a survey of EPAA member companies. That survey showed that the most frequently utilised test method for acute oral toxicity testing – unless others were specified by particular regulators – was the OECD 423 (Acute Toxic Class) guideline, which typically uses 6 to 7 animals.

Around the world, acute toxicity testing is generally required for agrochemicals, with testing called for via all three routes, often both for active substances and formulated products. It is also a normal requirement for chemicals, although most often via one route only (Europe's REACH regulation calls for two testing routes for substances produced in higher tonnages). For cosmetics it is either banned (i.e. in Europe) or not required in most parts of the world, though China and some countries in South America may still enforce it, including for finished products (A side issue here is that those countries that do require it do not always accept data from foreign countries, which can lead to double-testing.) The survey could find no specific requirements for food additives, flavouring and food-contact materials (such as packaging). Acute toxicity testing is not generally required for pharmaceuticals for human or veterinary use.

A major driver for the testing is for use in classification and labelling (C&L). EPAA members surveyed reported that this was the primary reason for carrying out such testing (regulatory driver). Use in C&L cuts across many sectors and countries, but in widely disparate ways. The maximum dose, for example, varies between 1g/kg body weight to 5g/kg depending on country and sector. The UN has tried to tackle this variability with its Globally Harmonised System (GHS), but as one speaker pointed out, in reality it is as yet neither global, nor harmonised.

Some companies did cite scientific drivers for doing the testing, although these were considered trivial given the lack of pathology and other scientific data gathered from these studies. Overall, the EPAA Task Force considered that the classical acute lethality studies were not needed to make C&L decisions, as there were other study types (such as the Fixed Dose Procedure [FDP; OECD TG 420], or dose escalation) that could be used instead.

The Task Force produced eight core conclusions and recommendations, and at the workshop in September it focused on the four highlighted below:

1. Before considering an acute toxicity test, all relevant information on a substance/product or similar compounds should be thoroughly evaluated to determine whether non-testing approaches could be used.
2. Regulators and policy makers worldwide should critically examine whether conventional approaches to acute toxicity testing could be replaced by non-lethal approaches for making C&L determination.
3. Requirements for acute dermal testing of chemicals and active substances in agrochemicals should be deleted.
4. Where tests could not be obviated, limits on doses should be reduced to at most 2g/kg, or preferably to 1g/kg.

In the final presentation of the morning session, the limitations of classification and labelling in providing useful information on acute toxicity to support the treatment and predict the outcome of cases of human poisoning of chemicals were discussed. Details of the outcome of an NC3Rs workshop which found that acute lethality studies are of little to no value in informing the clinical management of cases of overdose or poisoning were also presented<sup>2</sup>.

#### **4. Drivers for testing**

At the end of the morning session, the workshop split into two groups, each considering the same three questions:

1. Do participants concur with the conclusions of Seidle *et al.* (2010) regarding (lack of) scientific drivers for acute toxicity studies and those of Chapman *et al.* (2010) that acute toxicity studies have little value in supporting single accidental exposure/overdose?
2. Could alternative studies or study designs – ones that use lower doses/do not employ lethality as an endpoint – be used to meet regulatory needs such as classification and labelling?
3. Do participants concur that for scientific and animal welfare reasons the limit dose for acute toxicity studies in other sectors could be reduced to 1g/kg in line with pharmaceuticals?

The consensus was that:

1. The scientific drivers for acute lethality testing are very weak. However, some participants insisted that even though acute lethality testing provides little scientific knowledge, information (which may or may not involve direct

---

<sup>2</sup> Chapman, K *et al.* (2010) The value of acute toxicity studies to support the clinical management of overdose and poisoning: A cross-discipline consensus. *Regul Toxicol Pharmacol* 58, 354-359

testing) on the acute toxicity of substances it is needed to protect human health, in particular for workers. It was also acknowledged that specific information on acute toxicity has more value in data-poor situations than in data-rich situations.

2. EPAA survey. This found that the most commonly used study type was OECD TG 423. It was agreed that TG 420 is more humane and has the potential to provide dose response information. Active efforts should be made to promote acceptance of TG 420 globally.
3. Limit dose. The EU and OECD discourage testing above 2g/kg bodyweight, but some countries require testing up to 5g/kg. In the pharmaceutical sector limit doses for general toxicology have been reduced to 1g/kg. It was suggested that efforts could be made to challenge the use of doses up to 5g/kg. Questions were raised about how often systemic exposure increases when animals are administered doses above 1g/kg. One activity to consider would be collecting data on systemic exposure above 1g/kg.
4. It would be useful to organise follow-up workshops to address the following hypothetical situation in the pesticides and chemicals sectors: How could C&L be supported if acute toxicity testing were prohibited? What would be the consequence for downstream regulations? This may highlight opportunities for waiving requirements for acute toxicity testing in data rich situations.

## **5. Redundancy of multi route testing**

### **5.1 Presentation of concordance data**

Details of data analysis conducted by the UK Health and Safety Executive and Chemicals Regulation Directorate (formerly PSD) were presented demonstrating that acute dermal toxicity testing very rarely if ever provides information of value for hazard identification or assessment purposes when an acute oral study has been conducted<sup>3</sup>. The analysis compared the acute oral and dermal classifications for 240 active agrochemical ingredients and 438 chemicals, and found that just two agrochemicals and one chemical had a more severe classification by the dermal route. In all other cases the dermal classification was the same or less severe than the oral one. One of the agrochemical active substances with a more severe dermal classification produced marked local effects, causing burns on the skin.

The joint ECVAM and Humane Society International analysis of the scientific and regulatory value of multi-route testing, based on a data set of more than 2000 chemicals and agrochemical active substances tested via dermal and oral and/or

---

<sup>3</sup> Creton S *et al.* (2010) Acute toxicity testing of chemicals – Opportunities to avoid redundant testing and use alternative approaches. *Crit Rev Toxicol* 40(1), 50-83.

inhalation routes was presented. This analysis was built on the known concordance between data on oral and dermal testing, and set out to see whether inhalation testing in addition to oral testing might be considered redundant.

The overall dermal-oral concordance results were similar to those reported by the UK Health and Safety Executive and Chemicals Regulation Directorate. For chemicals, only one substance was classified more severely from dermal than from oral testing, while for pesticides the dermal test proved to be more sensitive for 6 substances. Oral testing resulted in a more severe classification than dermal for 43.9% of agrochemicals against 6.2% of chemicals studied. With inhalation studies the results were different. The overall inhalation-oral concordance went down to 24.1% in pesticides, as against 71.8% in chemicals. In particular, inhalation studies showed markedly higher classifications than oral studies – for 17% of chemicals and 51.7% of pesticides examined.

## **5.2. Potential implications for chemicals and agrochemicals**

There were two breakout groups, one to consider chemicals and one for pesticides. Four questions were asked to both groups.

The questions were:

1. Is there an adequate data set to support the removal of the requirement for acute dermal testing of chemicals/agrochemical active substances from relevant legislation, regulations and implementing guidance?
2. Is there an adequate data set to support the removal of the requirement for acute inhalation testing of chemicals/agrochemical active substances from relevant legislation, regulations and implementing guidance? If not, what data gaps need to be filled?
3. Can you envisage a situation where only one route (i.e. oral) of administration would be required for regulatory purposes, for example classification and labelling?
4. To what extent can the conclusions reached in relation to active substances be applied to agrochemical formulations? What are the limitations? What additional data/analyses should be considered?

The consensus was that:

- Existing data sets for oral/dermal concordance support the proposal that there should be no systematic requirement for testing by the dermal route, either for chemicals or for agrochemical active substances. Where information on acute toxicity by dermal route is necessary, this should be derived as much as possible from existing data, in particular from combining information on toxicity by oral route with toxicokinetic information. It should be explored

whether this could be incorporated into legislation e.g new biocides guidance and revisions to requirements for plant protection products and REACH

- Existing data sets *do not* support the same approach for inhalation testing where the concordance is not so good. However, the group felt that it would be necessary to avoid the situation of inhalation becoming a default second route under REACH. Waiving opportunities (e.g. consideration of physicochemical properties and the likelihood of exposure by the inhalation route) should be fully explored.
- Further work would need to address the lack of concordance for oral/inhalation e.g. relationship to physicochemical properties and/or chemical structure.
- It was acknowledged that especially in the area of pesticides, acute toxicity data for parent compounds is also used to trigger decisions on metabolites, which themselves will not all be tested for acute toxicity. In general, data poor and data rich situations need to be distinguished.
- It was suggested that TTC (Threshold of Toxicological Concern) and tiered strategies, possibly combined with (Q)SARs and *in vitro* methods, should be increasingly used to assess metabolites and impurities. The scientific rationale was supposed to be similar throughout sectors, though the triggers for testing might vary.

## **6. Recommended Next steps**

- To encourage/empower the ECVAM Scientific Advisory Committee (ESAC) to follow up redundancy of the dermal route: It was proposed that the new ESAC could review the concordance data and if appropriate produce a statement about the validity of the data and recommendations about how this could be applied in various sectors.
- In addition, the EPAA should approach ECHA with reference to the revision of REACH Annex VIII data requirements and/or the updating of REACH technical guidance to address the requirement for dermal testing; and to explore opportunities to feed into revisions to requirements for biocides and plant protection products in cooperation with relevant Commission Directorates General, industry associations and other key stakeholders.
- A workshop for the agrochemicals, and chemicals sectors focussing on how they could address C&L in the absence of acute toxicity testing. The workshop should also examine the downstream regulatory consequences of the removal of acute toxicity studies.
- To inform regulatory authorities in the United States, Canada, and other major markets about the findings and recommendations of the EPAA Acute Toxicity

Task Force and workshop, with the goal of enhanced international harmonisation in this area.

- To engage the EU commission partners to follow up the recommendations of Seidle et al. (2010) and of the EPAA workshop with individual regulators within EU member states.
- Further work would need to address the lack of concordance for oral/inhalation e.g. relationship to physicochemical properties and/or chemical structure.

## Appendix 1 – List of Participants

**Michel BOUVIER D'YVOIRE**

European Commission  
DG Enterprise and Industry

**Kees BREKELMANS**

European Commission  
DG Enterprise and Industry

**Magda CHLEBUS**

European Federation of Pharmaceutical  
Industries and Associations (EFPIA)

**Stuart CRETON**

National Centre for the Replacement,  
Refinement and Reduction of Animals in  
Research (NC3Rs)

**Lesley EARL**

Huntingdon Life Sciences

**Laura FABRIZI**

European Commission  
DG Health and Consumers

**James GERHART**

Merial Ltd.

**Krisztina GRANER**

National Institute of Chemical Safety  
Hungary

**Rob GUEST**

Harlan Laboratories Ltd.

**Helena HEMMING**

AstraZeneca

**Tom HOLMES**

Agchem Project Consulting Ltd. (APC)

**Ian INDANS**

Health and Safety Executive  
UK

**Utta JENSEN-KORTE**

European Commission  
DG Enterprise and Industry

**Zuzana KLOSLOVA**

Centre for Chemical Substances and  
Preparations  
Slovak Republic

**Pia KORJUS**

European Chemical Agency

**Catherine LECERF**

European Federation of Pharmaceutical  
Industries  
and Associations (EFPIA)

**Irene MANOU**

European Partnership for Alternative  
Approaches to Animal Testing (EPAA)

**Vanessa NIOT**

French Agency for food, environmental and  
occupational health safety (ANSES)

**Martin PAPARELLA**

Federal Environment Agency  
Austria

**Pilar PRIETO**

European Centre for the Validation of  
Alternative Methods (ECVAM)

**Inese PUZULE**

Latvian Environment, Geology and  
Meteorology Centre

**Kirsty REID**

Eurogroup for Animals

**Jon RICHMOND**

Animals Scientific Procedures, Home Office

**Maritsella RUBBIANI**

National Center for Chemicals  
Italy

**Sally ROBINSON**

AstraZeneca  
Acute Toxicity Task Force Chair

**Juia SCHEEL**

Henkel

**Andreas SCHNURSTEIN**

Evonik Industries

**Troy SEIDLE**

Humane Society International

**Yuhij TAQUAHASHI**

National Institute of Health Sciences  
Japan

**Manuela TIRAMANI**

European Food Safety Authority (EFSA)

**Sylvie TISSOT**

French National Institute for Industrial  
Environment and Risks (INERIS)

**Ton VAN HUYGEVOORT**

NOTOX

**Geert VERSTEGEN**

Belgian Poison Centre

**Martin WILKS**

Swiss Centre for Applied Human Toxicology

**Nicole ZERAFA**

Malta Standards Authority



The European Partnership  
for Alternative Approaches to Animal Testing

## Appendix 2 : Programme

### Acute Toxicity Testing Across Sectors Workshop

16 September 2010 - 08:30 to 17:30

Silken Berlaymont Hotel  
Boulevard Charlemagne 11 - 1000 Bruxelles

08:30 - 09:00	Registration and coffee	
09:00 - 09:15	Welcome and objectives of the workshop	<b>Sally ROBINSON</b> Acute Toxicity Task Force Chair, AstraZeneca
09:15 - 09:45	Cross-sector review of drivers for acute systemic toxicity testing	<b>Troy SEIDLE</b> Humane Society International
09:45 - 10:15	The value of acute toxicity data in classification and labelling	<b>Martin WILKS</b> Swiss Centre for Applied Human Toxicology, University of Basel
10:15 - 10:30	Coffee break	
10:30 - 11:45	Break-out groups	<b>Group 1</b> <b>Chair :Lesley EARL</b> (HLS, UK) <b>Rapporteur: Julia SCHEEL</b> (Henkel):  <b>Group 2</b> <b>Chair: Alan BOOBIS</b> (Imperial College, UK): <b>Rapporteur: Stuart CRETON</b> (UK NC3Rs):
	Feedback from breakout groups	
12: 30 - 13:45	Lunch	
13:45 - 14:15	Redundancy in acute toxicity testing: Oral vs dermal concordance	<b>Ian INDANS</b> UK Health and Safety Executive
14:15 - 14:45	Redundancy in acute toxicity testing: ECVAM/HSI data set	<b>Pilar PRIETO</b> ECVAM
14:45 - 15:00	Coffee break	
15:00 - 16:15	Break-out groups	<b>Group 1 (Pesticides)</b> <b>Chair: Dr Manuela TIRAMANI</b> (Pesticide Risk Assessment European Food Safety Authority) <b>Rapporteur : Martin WILKS</b> (Swiss Centre for Applied Human Toxicology, University of Basel)  <b>Group 2 (Chemicals)</b> <b>Chair: Robert GUEST</b> (Harlan, UK) <b>Rapporteur: Tom HOLMES</b> (APC)
16:15- 17:00	Feedback from break-out groups and next steps	
17:00 - 17:15	Summary and conclusions	<b>Sally ROBINSON</b>
17:30	End of the meeting	