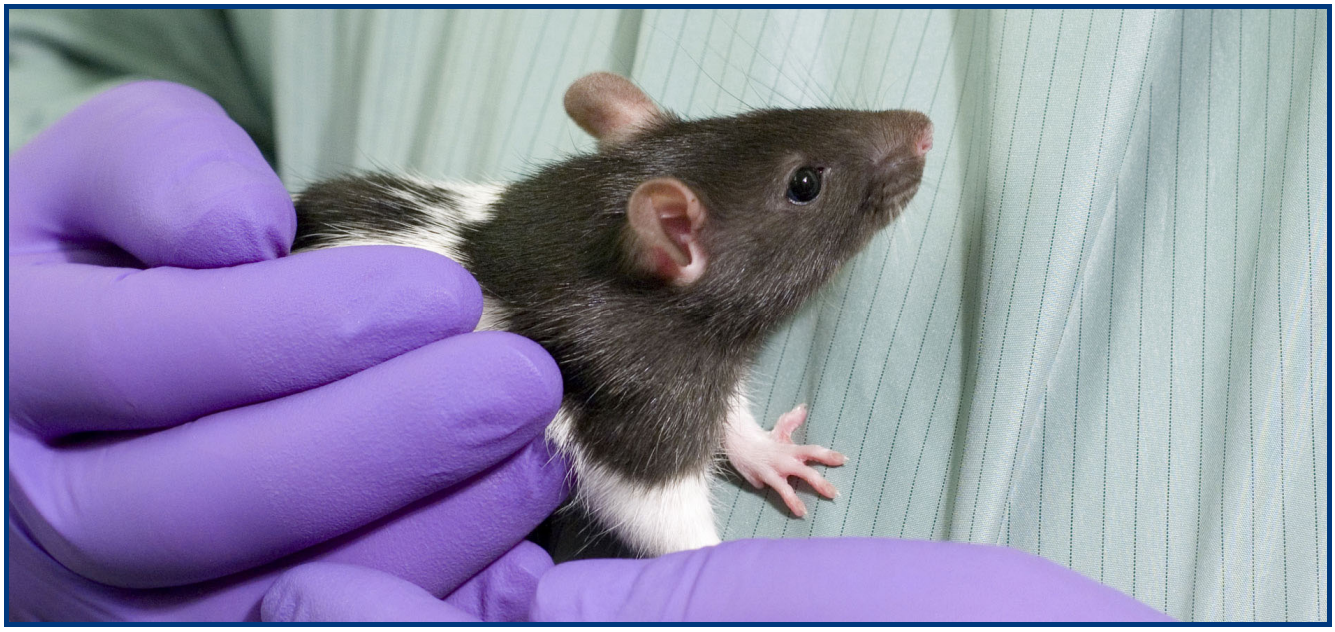
National Centre for the Replacement, Refinement
and Reduction of Animals in Research

Challenging the regulatory requirement for acute toxicity studies in the development of new medicines

A workshop report



by Kathryn Chapman, NC3Rs; Sally Robinson, AstraZeneca

Please note: This workshop report does not necessarily reflect the views of individual companies and organisations that attended.

Contents

The 3Rs - definitions

Replacement: methods which avoid or replace the use of animals in an area where animals would otherwise have been used.

Reduction: methods which minimise animal use and enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals, thereby reducing future use of animals.

Refinement: improvements to husbandry and procedures which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare.

1 Introduction

International regulations relating to human health require that all new pharmaceutical drugs are tested for their safety prior to their use in human volunteers and patients. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used. These studies are usually conducted in rodents and this test is particularly contentious as it is the only test in pharmaceutical development where lethality is a key endpoint. Increasingly, there is also controversy about the scientific value of the data obtained and its correlation with predicting acute toxic effects in humans, particularly when compared with the suffering caused to the animals used.

Definition of acute toxicity: Acute toxicity is that produced after administration of a single dose (or multiple doses) in a period not exceeding 24 hours, up to a limit of 2000 mg/kg

Objective of acute toxicity studies: To identify a dose causing major adverse effects and an estimation of the minimum dose causing lethality, according to regulatory guidelines (see Table 1). ICHM3 suggests these studies or suitable alternatives are required prior to the first administration of a new medicine in humans.

Box 1 Acute toxicity studies

This report only covers conventional acute toxicity studies conducted to support the development of new medicines, where lethality is an endpoint. Acute toxicity testing of chemicals and intermediates by the pharmaceutical and chemical industries is not covered, because it is done for a different purpose and under different regulatory guidance. Throughout the report, acute toxicity refers to the studies done for the objective described above (Box 1) under the regulatory guidance outlined in Table 1. Extended single dose studies that specifically support single dose in humans (e.g. microdosing[1]) are not the subject of this report.

To evaluate the utility of acute toxicity data and possible alternatives, an expert working group, coordinated by the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), and comprising 15 European pharmaceutical companies and contract research organisations, has been established (see Appendix 1 for membership).

The working group's objectives are two-fold. Firstly, the short-term objective is to agree an approach to conventional single dose acute toxicity studies that focuses on **reduction** and **refinement** and secondly in the longer term the **replacement** of these studies while ensuring that the safety of humans is maintained. Fundamental to these objectives is the timing of acute toxicity studies and whether they are necessary prior to the first administration of a new medicine in humans, also known as first-in-man (FIM) studies, or only later, when they are used to predict overdose levels in man.

Critically, if acute toxicity studies are only deemed necessary to predict overdose effects then they do not need to be conducted prior to FIM studies but only when the drug is more freely available and therefore at higher risk of overdose occurrences (e.g. Phase 3 or registration). A shift in the timing of acute toxicity tests alone would have a significant impact on animal use because many drugs fail during clinical development after FIM and are dropped from development. Such a shift would result in acute toxicity tests being carried out on fewer compounds.

By taking an evidence-based review of the value of acute toxicity studies before FIM, and sharing and analysing data, the working group has demonstrated that the number of animals used can be significantly reduced, and that ultimately these studies could be phased out completely.

As part of this collaborative initiative, the NC3Rs held a workshop in November 2006 to discuss the working group's findings with an audience of regulators and toxicologists from Europe, the USA and Japan. This report describes the workshop and provides a background to the use of animals in acute toxicity studies in the pharmaceutical industry and the approach taken to influence change both within companies and in regulatory guidance.

2 Background

2.1 What are acute toxicity studies?

Acute toxicity studies for pharmaceutical development involve the administration of a single dose of test compound to two different mammalian species, often by two different routes (Table 1). These are usually the clinical route and an additional parenteral route (usually intravenous) to ensure systemic exposure. Unless the clinical route is intravenous, in which case, only the intravenous route is tested. The routes of administration and species required vary according to the regulatory authority. Administration of the compound is usually followed by 14 days of observation including the recording of clinical signs (e.g. behaviour, body weight), duration, and reversibility of the toxic effect.

2.2 Why are acute toxicity studies performed?

Regulatory authorities require data from acute toxicity studies for the registration of any pharmaceutical intended for human use. Traditionally, the information obtained from these studies has been used to set an appropriate dose level for repeat dose studies in animals and to support the effects of overdose in humans. They may also be used to support doses for FIM studies and to give an early indication of target organ toxicity[3].

	EEC[2]	**US[3]**	**JAPAN[4]**
Species	2*	2 (1 non-rodent)	2 (1 non-rodent)
Routes	2** clinical + another ensuring exposure	2** (as EEC)	1 Clinical route
Days of Observation	7-14	14	14

* usually rat and mouse
** only study type where a second route (intravenous) is routinely required
US/Japan: Dose-escalation is an acceptable alternative for non-rodents;
no region mentions dose-escalation as alternative for rodents

Table 1: Regulatory framework

3 Progress to date: inter-company data sharing

To review the current use of acute toxicity studies, questionnaires were completed by the members of the working group to determine the reasons for conducting acute toxicity studies and the type of study design, including the number of animals used. The results of this survey indicated a large inter-company variation in approach and demonstrated there was scope for agreeing best practice and reducing animal numbers. This initiated discussions around the fundamental issue of whether companies actively used the data generated in acute toxicity studies or whether they were primarily conducted to fit regulatory requirements.

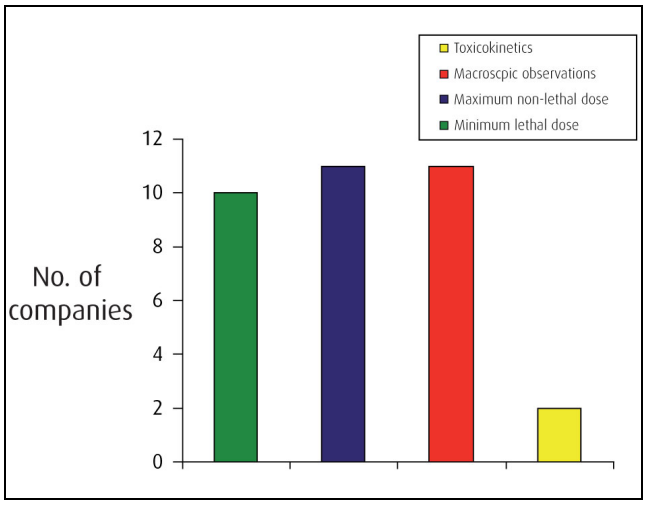


Figure 1: Information gathered from conventional acute toxicity studies

3.1 Preclinical value of acute toxicity studies

Results from the questionnaire showed that the majority of companies only used the studies to calculate the minimum lethal dose and maximum non-lethal dose (Figure 1). Pathology is usually limited to macroscopic observations so that target organs are generally not identified. There is no clinical pathology or measure of exposure in acute toxicity studies. Dose levels for repeat dose studies are determined from other study types such as dose range finding and dose escalation studies. This is supported by the fact that many companies run acute toxicity studies in parallel with one month repeat dose studies.

The results from the questionnaire provide compelling evidence to suggest that there is little preclinical reason in carrying out acute toxicity studies prior to FIM.

3.2 Value of acute toxicity studies prior to FIM studies

The working group have shared information on 40 different compounds that had progressed through development and were given in FIM studies. The results and recommendations were discussed at the workshop and will form the basis of a peer-reviewed publication to be submitted in 2007[5]. For more detail on this data-sharing exercise please contact Sally Robinson (sally.robinson@astrazeneca.com). The output clearly demonstrated that acute toxicity studies should not be required prior to FIM studies.

3.3 Reduction in animal numbers used in acute toxicity studies

A review of the number of acute toxicity studies in 2006 indicates that, in the absence of regulatory change, most companies involved in the working group have reduced the number of studies carried out per drug since the start of the initiative (Figure 2). By sharing information on study design between the companies, significant progress has also been made in reducing the number of rats and mice used in each study (Figures 3 and 4).

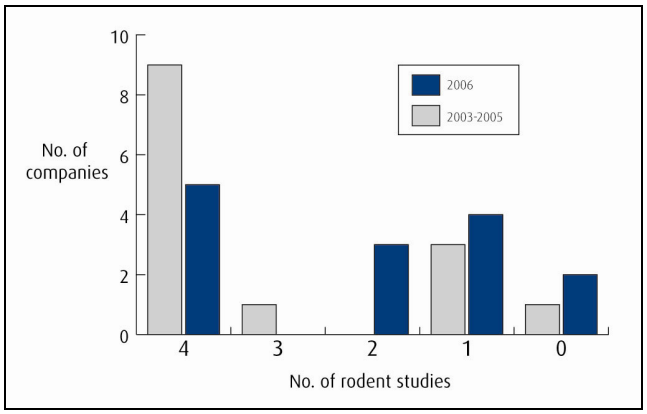


Figure 2: Number of rodent studies conducted per investigative compound in 2003-2005 compared to 2006

The maximum number of studies conducted by companies was four, which accounted for acute toxicity tests in two species using two different routes of administration. On the basis of the working group sharing best practice, many companies moved away from carrying out the full four studies, leading to a reduction in the number of studies conducted per compound.

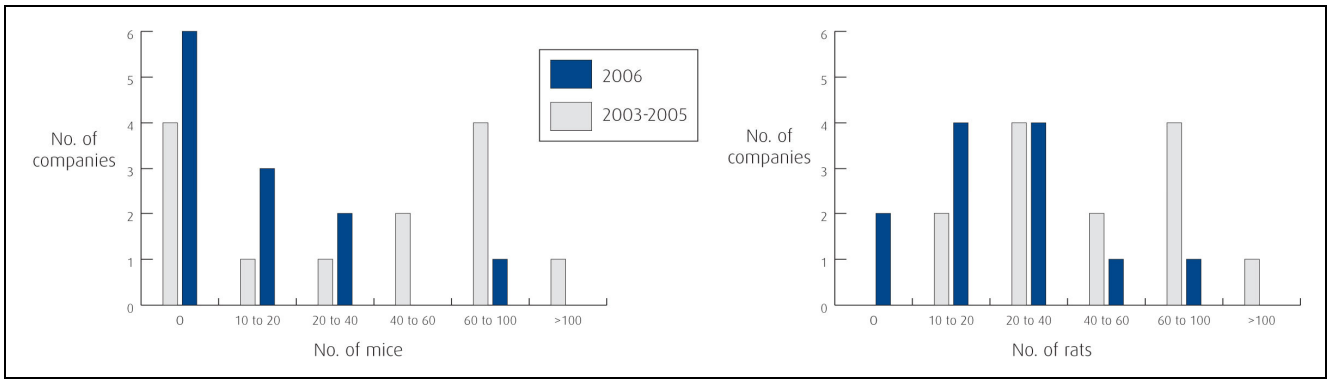


Figure 3: Number of mice used per compound in each company
The sharing of data and experience has led to a reduction in the number of mice used per conventional acute toxicity study. In 2003-2005 seven companies were using from 40 to over 100 mice per study. In 2006, since the working group shared best practice, most companies use less than 40 mice and more companies use no mice at all.

Figure 4: Number of rats used per compound in each company
The sharing of data and experience has led to a reduction in the number of rats used per conventional acute toxicity study. In 2003-2005 seven companies were using from 40 to over 100 rats per study. In 2006, since the working group shared best practice, most companies use less than 40 rats and two companies use no rats at all.

3.4 Clinical value of acute toxicity studies

The question of whether acute toxicity studies are of value in predicting human overdose is controversial. At high doses of a drug or following intravenous dosing, rodents often die with fairly non-specific effects that may have no relevance for the human overdose situation. It is difficult to manage acute poisoning in humans when a drug is first marketed as at this point clinical overdose data will be extremely limited. Animal data from toxicity studies is sometimes the only information available to clinicians in supporting human overdose effects. However, whether clinicians use acute toxicity data when treating overdose patients is debateable. In order to address this, the working group is collaborating with the Lyon Poison Centre to review whether rodent acute toxicity data predicts the effects of overdose in humans and whether information from other studies with functional assessments e.g. safety pharmacology studies might be more useful.

These questions are currently being investigated by the working group.

4 Workshop

A workshop, attended by 50 participants from Europe, the US and Japan, including representatives from the pharmaceutical industry, contract research organisations, and national and international regulatory bodies was held in November 2006 in London (Appendix 2). The purpose of the workshop was to discuss with a broader audience the results of the data sharing exercise undertaken by the working group and possible opportunities for influencing regulatory change for acute toxicity studies.

The workshop comprised of presentations on the regulatory environment from the European Agency for the Evaluation of Medicinal Products (EMEA), the American Food and Drug Administration (FDA), and the Japanese Ministry of Health, Labour and Welfare (MHLW), followed by break-out groups to discuss developing pharmaceutical drugs without data obtained from acute toxicity studies.

ICHM3 is the international regulatory guideline that covers the timing of non-clinical safety studies including acute toxicity studies (Box 2)[6]. The guideline is currently undergoing revision and therefore the workshop was a timely opportunity to discuss opportunities for applying the 3Rs with regulators.

> The acute toxicity of a pharmaceutical should be evaluated in two mammalian species prior to the first human exposure. A dose escalation study is considered an acceptable alternative to the single dose design.

Box 2: Current ICHM3 regulatory guideline[6]

The recommendations of the working group were supported by representatives from the regulatory bodies. This included support for the proposal that information from other studies, such as appropriately conducted non-GLP dose escalation studies or short duration dose ranging studies that define a maximum tolerated dose, could be used prior to FIM rather than acute toxicity data, and that investigations may be limited to the clinical route only.

Discussion indicated that the recommendations made by the working group are likely to be reflected in the new draft ICHM3 guidance.

4.1 Breakout sessions

Delegates were asked to consider a range of questions relating to the preclinical assessment of acute toxicity of a hypothetical compound. Qualitative information arising from the discussion was supplemented by a quantitative survey of delegates at the end of the meeting. The results of this are presented in italics. 35% of the respondents indicated that their views had changed based on the evidence presented at the workshop and the views of an additional 47% of respondents were reinforced or they remained convinced that acute toxicity tests were not necessary prior to FIM.

4.1.1 What do you perceive the data from acute toxicity studies are used for?

There was a consensus from the participants that acute toxicity studies were never used to identify target organ toxicity and that data could be obtained from other studies that were being performed.

100% of respondents found data from acute toxicity studies of little or no use and only used the information in dose setting for other studies in exceptional circumstances.

100% of respondents agreed that acute toxicity studies were not used to identify target organs.

100% of respondents never use acute toxicity data to help set the starting dose in man. (Extended single dose studies in animals to support microdosing in humans are covered by separate regulatory guidance. They are not conducted to the same design as acute toxicity studies (e.g. lethality is not an endpoint) and generate all the standard toxicological data that would support dosing in humans.)

81% of respondents thought the data obtained from acute toxicity studies was of no use to regulators or clinicians.

100% of respondents agreed that they would not carry out acute toxicity testing if it were not a regulatory requirement.

4.1.2. How are acute toxicity data used to support human safety alongside other preclinical data?

There was scepticism from the clinicians present about the usefulness of acute toxicity data, since information on lethality was of limited use and that what was actually required was detail of target organ toxicity, the duration of adverse effects and potential antidotes. Information on overdose could be provided instead from dose escalation and preliminary single dose studies.

There may be rare circumstances where single dose acute toxicity studies could be useful. For example, where the no observed adverse effect level (NOAEL) in repeat dose studies was lower than anticipated; single dose data may provide additional support for single doses in humans. However, this would only be useful if the test included pathology and other additional endpoints, which it currently does not.

4.1.3 What other study types could be used to get information on high single dose toxicity?

The following study types and designs were proposed as alternatives:

- single dose tolerability for *in vivo* genetic toxicology e.g. micronucleus
- other preliminary single dose tolerability studies
- pharmacology
- safety pharmacology
- dose range finding

4.1.4 If acute toxicity studies are performed, what is the added scientific value of more than one rodent species and the use of a route in addition to the clinical route?

The opinion was expressed that if acute toxicity data in one rodent species was flawed, then what justification was there for using two rodent species? In principle, the recommendation of using two mammalian species covers the possibility of different species sensitivity. It is hard to draw conclusions however when there are a lack of published data comparing mouse with rat acute toxicity data. Some data were described at the workshop that showed only a 2-fold difference between mice and rats in LD50 studies of various chemicals (with the exception of warfarin), supporting the suggestion that the use of two rodent species is not necessary. This supports initial observations from the working group's data sharing exercise[6]. There was no perceived added value of using two rodent species or of using more than one route of administration, except in cases where high levels of exposure could not be achieved through the clinical route. But in such a case the alternative route ensuring exposure might be the only route required.

94% of respondents agreed that the use of more than one rodent species (mouse and rat) was of no value and 88% agreed that only one clinical route was necessary.

100% of respondents agreed that they would not use lethality as an endpoint unless it was a regulatory requirement.

4.1.5 If you had to do acute toxicity for regulatory purposes only, what would you see as the minimum requirement and at what point in the development process?

There was general agreement that if tests were only being done for a regulatory purpose with no sound scientific justification then they should not be conducted. The minimum package was considered to be one rodent species, probably the rat, administered via the clinical route prior to phase 3 clinical trials only.

4.1.6 What is the added scientific value of carrying out an acute toxicity study prior to FIM?

There was general agreement that acute toxicity studies prior to FIM were of no use provided that the high dose was evaluated in other short term study types.

100% of respondents agreed human safety would not be compromised if acute toxicity studies were not carried out.

4.1.7 Have you used acute toxicity data to predict overdose effects in humans?

Some participants used these data to decide whether child-proof packaging was necessary for the drug. However, whether acute toxicity studies from rodents provide the best information to base this decision on was disputed. Comprehensive safety pharmacology studies (such as those described in 4.2.3) were considered to be

more informative. To date, there are no published data available comparing the preclinical animal data and clinical data for acute toxicity. Without this information, there was some caution expressed about accepting that acute toxicity studies were never useful in making decisions on child-proof packaging and overdose effects. These questions will be addressed in the collaboration with the Lyon Poisons Centre.

5 Discussion

Acute toxicity studies are controversial in terms of the questionable value of the data obtained compared with the suffering caused to the animals used. Although progress has been made, for example in abandoning the requirement for LD50 studies for pharmaceuticals, the regulatory requirement for acute toxicity remains.

It is clear that the strategy for toxicity testing has changed significantly over the years in order that early toxicology information can help support decisions on the best compounds to progress as potential human medicines. Acute toxicity tests are no longer the first tests performed and the data they provide can be obtained from other studies.

The approach taken by the working group has demonstrated the value of sharing and collating data in order to harmonise study designs, implement the 3Rs, and provide evidence to support regulatory change. By bringing together representatives from the pharmaceutical industry and the regulatory bodies to discuss the output of the working group it is clear that considerable progress can be made in supporting and facilitating the removal of the need to perform acute toxicity studies prior to FIM studies.

While this is an important step, there is more that can be achieved in terms of ensuring that, where acute toxicity studies are required, the harmonised and reduced study design recommended by the working group is adopted by all companies.

Establishing whether acute toxicity studies are required for predicting human overdose is a critical next stage and the collaboration with the Lyon Poison Centre will be pivotal in determining whether acute toxicity studies are needed at all to ensure human safety.

This initiative illustrates the benefits that can be achieved in terms of implementing the 3Rs by a coordinated approach and sharing data to reach a common position based on evidence and science. It is important to consider where else a similar strategy could be applied to enhance the application of the 3Rs.

6 Conclusion

The conclusion is that acute toxicity data are:

1. Extremely limited, concentrating on minimum lethal and maximum non-lethal doses.

2. Of less use than other, less harmful, animal tests that are superior for deciding appropriate doses for further animal studies.

3. Not particularly useful for information on the nature of toxic effects, which are better evaluated in other routine studies.

4. Not, in practice, used to set doses in the first human clinical trials because other routine studies provide more informative data.

7 References

1. CPMP/SWP/2599/02/Rev 1 23 June 2004: Position paper on non-clinical safety studies to support clinical trials with a single microdose

2. Annex 1: Directive 2001/83/EC of the European Parliament (6 Nov 2001, see page 44)

3. CDER Guidance for Industry: Single dose acute toxicity testing for pharmaceuticals (Aug 1996)

4. ICH Japan, Guidelines for new drug registration with harmonized tripartite guidelines, Yakuji Nippo Ltd. (1999)

5. Sally Robinson et al in preparation

6. ICH M3: Guidance on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals (1997)

Appendix 1: Working group members

Sally Robinson (Chair)	AstraZeneca
Kathryn Chapman	NC3Rs
Jean-Luc Delongeas	Servier
Elizabeth Donald	Charles River Laboratories
David Dreher	Covance
Matthias Festag	Roche
Sophie Kervyn	Eli Lilly and Company
Ann Lampo	Johnson & Johnson
Kamil Nahas	Pfizer
Vincente Nogues	Novartis
Deborah Ockert	ALTANA Pharma AG
Sally Old	Sanofi-Aventis
Nigel Pickersgill	MDS Pharma Services
Kev Somers	GlaxoSmithKline
Claudia Stark	Schering
Peter Stei	Boehringer Ingelheim

Additional members who joined after the November 2006 workshop:

Huntingdon Life Sciences

Aptuit

UCB

Acute Toxicity Workshop

27 November 2006, Central London

Agenda

09.30 – 10.00	REGISTRATION and COFFEE
10.00 – 10.10	Welcome and introduction
10.10 – 10.20	NC3Rs - Background and industry initiatives
10.20 – 10.50	European Pharmaceutical Company Initiative Challenging the Requirement of Acute Toxicity Studies in Rodents
10.50 – 11.20	Feasibility study: the value of acute rodent studies in supporting overdose in man
11.20 – 11.40	A regulatory perspective on Rodent Acute Toxicity studies (EMEA)
11.40 – 12.00	COFFEE
12.00 – 12.20	Acute toxicity testing - FDA perspective
12.20 – 12.40	A MHLW perspective on Rodent Acute Toxicity studies
12.40 – 13.25	LUNCH
13.25 – 13.35	Introduction to afternoon session
13.35 – 14.55	Break out group discussions
14.55 – 15.15	COFFEE
15.15 – 16.15	FEEDBACK AND DISCUSSIONS
~ 16.15	CLOSE

Appendix 3: Attendees of workshop

Attendees:

ALTANA Pharma AG
Aptuit
Association of the British Pharmaceutical Industry (ABPI)
AstraZeneca
Boehringer Ingelheim
Canadian Council on Animal Care
Charles River Laboratories
CIT Safety & Health Research Laboratories
Covance
European Agency for the Evaluation of Medicinal Products (EMEA)
Eli Lilly and Company
Federal Institute for Drugs and Medical Devices (BfArM)
ForthTox Limited
GlaxoSmithKline
Huntingdon Life Sciences
J.A. Reynolds & Associates
Johnson & Johnson
Lyon Poison Centre
MDS Pharma Services
Medicines and Healthcare Products Regulatory Agency (MHRA)
Merck & Co.
National Institute of Health Sciences
National Institute of Pharmaceuticals and Medicines (INFARMED)
Novartis
Organon
Pfizer
Roche
Sanofi-Aventis
Schering
Sequani Limited
Syngenta
UK Animal (Scientific Procedures) Inspectorate
US Food and Drug Administration (FDA)