Regulatory Toxicology and Pharmacology 70 (2014) 413–429

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Recommendations from a global cross-company data sharing initiative on the incorporation of recovery phase animals in safety assessment studies to support first-in-human clinical trials

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ARTICLE INFO

Article history:
Received 14 July 2014
Available online 29 July 2014

ABSTRACT

An international expert group which includes 30 organisations (pharmaceutical companies, contract research organisations, academic institutions and regulatory bodies) has shared data on the use of recovery animals in the assessment of pharmaceutical safety for early development. These data have

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http://dx.doi.org/10.1016/j.yrtph.2014.07.018
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Keywords:
Recovery
Reversibility
3Rs
Reduction
Non-clinical
Repeat-dose studies
Regulatory toxicology
Rodent
Primate
Dog

been used as an evidence-base to make recommendations on the inclusion of recovery animals in toxicology studies to achieve scientific objectives, while reducing animal use.

Recovery animals are used in pharmaceutical development to provide information on the potential for a toxic effect to translate into long-term human risk. They are included on toxicology studies to assess whether effects observed during dosing persist or reverse once treatment ends.

The group devised a questionnaire to collect information on the use of recovery animals in general regulatory toxicology studies to support first-in-human studies. Questions focused on study design, the rationale behind inclusion or exclusion and the impact this had on internal and regulatory decisions. Data on 137 compounds (including 53 biologicals and 78 small molecules) from 259 studies showed wide variation in where, when and why recovery animals were included. An analysis of individual study and programme design shows that there are opportunities to reduce the use of recovery animals without impacting drug development.

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1. Introduction

1.1. Background

It is a scientific, ethical and regulatory requirement that before any potential new medicine can be administered to humans its safety must be adequately assessed in animals in order to inform safe starting doses and clinical safety monitoring for human studies. To meet this objective, repeat-dose toxicology studies in rodents and/or non-rodents are typically required by global regulatory agencies before a drug can be approved for administration to humans. These studies aim to characterise the toxicological profile of the test compound following repeated administration, including identification of potential target organs of toxicity and exposure/response relationships. This is generally achieved through the use of three dose groups (i.e. to test low, intermediate and high levels of the drug), plus a control group. Additional animals are frequently included in these studies to evaluate the reversibility or recovery of any toxicities observed during the dosing phase. Once treatment is complete, these additional animals are retained 'off-dose' for a predetermined period so that recovery can be assessed. The 'recovery phase' should be of sufficient duration for the drug to clear from circulation, and/or disengage from its receptor target, and be of adequate time to determine whether the effects observed during the treatment phase persist, or are partially or fully reversible. Demonstration of full or partial reversibility of toxicity can then be used as part of the overall assessment of the suitability of the drug for administration to humans. In rare cases, new toxicities may also be identified during the recovery phase (i.e. delayed toxicity).

Although it is a regulatory expectation that recovery from toxic effects will be considered at some point during the drug development process, this is not necessarily required prior to first-in-human (FIH) clinical trials and does not necessarily require the use of dedicated recovery animal groups. Guidance on the inclusion of recovery animals to support clinical development phases and the associated regulatory recommendations is given in the International Conference on Harmonisation (ICH) guidelines, ICH, M3(R2)*Non-clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* and the recently published accompanying *Question and Answer* document (ICH, M3(R2), ICH, M3(R2) Q&A). Guidance on the use of recovery animals for particular classes of drug include ICH S6(R1), *Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals* and ICH S9, *Nonclinical Evaluation for Anti-cancer Pharmaceuticals* (ICH, S6(R1), ICH, S9). Relevant extracts from these guidances are set out in Tables 1 and 2.

Typically, reversibility is assessed through the inclusion of additional 'recovery' animals on toxicology studies at different stages during the development programme. However, depending on the specific study objectives and the nature of the observed changes, the addition of recovery animals may not always be necessary to determine whether a toxic effect is reversible, and in many cases an evaluation of reversibility based on scientific assessment alone may be sufficient. For example, in cases where the lesions are known to be reversible, or where toxicities occur at clinically irrelevant exposure levels, demonstration of reversibility by inclusion of recovery animals may not always be necessary or justifiable. Generally, where recovery animals are considered important, they are only needed to evaluate a particular toxicity or lesion once during a development programme and on one clinically relevant dose group. The ICH M3(R2) Question and Answer document (ICH, M3(R2) Q&A) (Table 2) gives examples of occasions where inclusion of recovery animal groups may be appropriate, such as where there is severe toxicity at clinically relevant exposures. However, the guidance is intentionally flexible and does not provide specific information on when it is appropriate to include recovery animals during the drug development process.

A number of publications have examined the use of recovery animals in non-clinical toxicology studies, with a focus on the typical study designs used, including the number of dose groups that included recovery animals and the number of recovery animals per group (Baldrick, 2008; Baldrick, 2011; Brennan et al., 2010; Chapman et al., 2009; Chapman et al., 2012; Chapman et al., 2010; Clarke et al., 2008; Konigsson, 2010; Lynch et al., 2009; Pandher et al., 2012; Perry et al., 2013; Smith et al., 2005; Sparrow et al., 2011). Many of these make recommendations for appropriate study designs, stating that inclusion of recovery animals should follow a study/project specific science-driven approach to comply with the regulatory expectations whilst minimising laboratory animal use. However, the majority of the published literature focuses on studies for biological drugs conducted in non-human primates (NHPs), and/or evaluate inclusion of recovery groups for individual studies, rather than inclusion in the development package of new molecules as a whole.

Opinions and practices vary around why, when and how recovery animals should be included on toxicology studies. Given this uncertainty, recovery animals may be included by default without specific consideration of their value or scientific utility, to reduce potential perceived regulatory hurdles and/or to prevent repeating a toxicology study if unexpected toxicity is observed that might necessitate further evaluation of reversibility to support dosing in humans. The main reason for inclusion of recovery animals is to address the reversibility of an effect seen in an earlier study. However, there are also scientific reasons for the inclusion of these additional 'off-dose' animals which may not directly assess reversibility, such as to inform clinical dosing, to assess the potential for delayed toxicity, and/or to gather data on immunogenicity or PK/PD relationships. Although these reasons may not fall within the regulatory expectations with regards to the assessment of reversibility, these are examples of how 'off-dose' animals may be used

Table 1
International Conference on Harmonisation general guidance regarding the need to assess recovery/reversibility on non-clinical studies to support clinical development.

ICH M3 (R2): Conduct of Nonclinical Safety Studies
D. General principles (1.4) "The goals of the non-clinical safety evaluation generally include a characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility."
Table 3. Recommended non-clinical studies to support exploratory clinical trials
Footnote c. "Generally, extended single dose toxicity studies should be designed to evaluate hematology, clinical chemistry, necropsy, and histopathology data (control and high dose only if no treatment-related pathology is seen at the high dose) after a single administration, with further evaluations conducted 2 weeks later to assess delayed toxicity and/or recovery."
Footnote d. "A single dose level to assess reversibility/delayed toxicity on day 14 can support the microdose approach. The dose level used need not be the high dose but should be a dose that is at least 100 times the clinical dose."
ICH S6 (R1) Pre-clinical safety evaluation for biotechnology-derived pharmaceuticals
Part I
4. Specific considerations
4.4. Repeat dose toxicity studies "A recovery period should generally be included in study designs to determine the reversal or potential worsening of pharmacological/toxicological effects, and/or potential delayed toxic effects. For biopharmaceuticals that induce prolonged pharmacological/toxicological effects, recovery group animals should be monitored until reversibility is demonstrated"
Part II
3. Study design
Section 3.3 Recovery "Recovery from pharmacological and toxicological effects with potential adverse clinical impact should be understood when they occur at clinically relevant exposures. This information can be obtained by an understanding that the particular effect observed is generally reversible/non-reversible or by including a non-dosing period in at least one study, at least one dose level, to be justified by the sponsor. The purpose of the non-dosing period is to examine reversibility of these effects, not to assess delayed toxicity. The demonstration of complete recovery is not considered essential. The addition of a recovery period just to assess potential for immunogenicity is not required."
ICH S9 Nonclinical evaluation for anti-cancer pharmaceuticals
II. Studies to support non-clinical evaluation
D. General toxicology (2.4) "Assessment of the potential to recover from toxicity should be provided to understand whether serious adverse effects are reversible or irreversible. A study that includes a terminal non-dosing period is called for if there is severe toxicity at approximate clinical exposure and recovery cannot be predicted by scientific assessment. This scientific assessment can include the extent and severity of the pathologic lesion and the regenerative capacity of the organ system showing the effect. If a study of recovery is called for, it should be available to support clinical development. The demonstration of complete recovery is not considered essential."
5. Notes "For non-rodent studies, dose groups usually consist of at least 3 animals/sex/group, with an additional 2/sex/group for recovery, if appropriate (see section II.D (2.4)). Both sexes should generally be used, or justification should be given for specific omissions."

by industry to address a particular scientific question. Additionally, the reasons for inclusion or exclusion of recovery animals may differ depending on the molecule class (i.e. the expectation of off-target toxicity for small molecules or the potential for prolonged PD effects for biologicals), the novelty of the target pathway (i.e. how much prior information is available regarding expected toxicities, based on pharmacological class or previous experience), as well as the intended therapeutic area or anticipated target organs.

This paper evaluates current practices for the use of recovery animals in order to develop recommendations on considerations for toxicology study design, with a focus on both small molecule and biological compounds. Clear opportunities to reduce animal use and resource have been identified. In brief, we recommend that decisions regarding when and if recovery animals are included on repeat-dose toxicology studies should be based on scientific considerations, rather than a default approach.

1.2. Working group objectives

The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) industry working group on recovery animals was launched in 2012 in collaboration with the Medicines and Healthcare Products Regulatory Agency (MHRA). The group shared data on the use of recovery animals in general regulatory toxicology studies in order to make evidence-based recommendations on where and why recovery animals should be included to optimise risk assessment for human studies while reducing animal use, with an initial focus on studies designed to support FIH clinical trials.

The group was represented by 19 global pharmaceutical companies, 3 contract research organisations (CROs), 2 academic institutions and 4 regulatory bodies (see author affiliations) and was managed and facilitated by the NC3Rs.

2. Methods

A pilot questionnaire was conducted to collect information on the use of recovery animals in general regulatory toxicology studies. Based on the breadth of results from this exercise the questionnaire was revised and restricted to pivotal repeat dose studies to support FIH clinical trials only (Tables 3A and 3B and Table S1). The questionnaire was therefore designed to examine the use and value of recovery animal groups in studies to support FIH only, and was not intended to evaluate the role of recovery animals at later stages in the development process. Criteria for studies suitable for inclusion in the questionnaire were set out by the working group as follows:

- pivotal studies to support FIH;
- good laboratory practice compliant;
- general toxicology repeat dose studies only and;
- studies carried out since January 2010 (to reflect the revision and addendum of ICH M3 and ICH S6 guidelines).

The questionnaire content was agreed upon by the working group in advance to ensure accurate interpretation of questions. Information on molecule type (e.g. small molecule or biological), therapy area and current clinical status were requested. Questions focused on study design (e.g. species, recovery duration and the number of animals used), the rationale behind inclusion or exclusion (case-specific *vs.* default inclusion), and the impact that this had on internal and regulatory decisions. Additional information considered useful to the analysis was provided in the comments field. A copy of the full questionnaire is provided in Supplementary Table S1.

To minimise the potential for bias, respondents were asked to start with their most recent compound and work backwards, systematically, towards January 2010. Data were collected on studies

Table 2
International Conference on Harmonisation M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals. Question and Answer document regarding appropriate assessment of reversibility.

ICH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals. Q & A.	
Q.	When is assessment of reversibility considered to be appropriate and is it important to demonstrate full reversibility or is it sufficient to demonstrate the potential for full reversibility?
A.	Evaluation of the potential for reversibility of toxicity (i.e., return to the original or normal condition) should be provided when there is severe toxicity in a nonclinical study with potential adverse clinical impact. The evaluation can be based on a study of reversibility or on a scientific assessment The scientific assessment of reversibility can include the extent and severity of the pathologic lesion, the regenerative capacity of the organ system showing the effect and knowledge of other drugs causing the effect. Thus, recovery arms or studies are not always critical to conclude whether an adverse effect is reversible. The demonstration of full reversibility is not considered essential. A trend towards reversibility (decrease in incidence or severity), and scientific assessment that this would eventually progress to full reversibility, are generally sufficient. If full reversibility is not anticipated, this should be considered in the clinical risk assessment A toxicity study that includes a terminal non-dosing period is generally warranted if a scientific assessment cannot predict whether the toxicity will be reversible and if: 1. there is severe toxicity at clinically relevant exposures (e.g., ⩽10-fold the clinical exposure) or 2. the toxicity is only detectable at an advanced stage of the pathophysiology in humans and where significant reduction in organ function is expected. (The assessment of reversibility in this case should be considered even at >10-fold exposure multiples.) A toxicity study that includes a terminal non-dosing period is generally not warranted when the toxicity: 1. can be readily monitored in humans at an early stage before the toxicity becomes severe or 2. is known to be irrelevant to humans (e.g., rodent Harderian gland toxicity) or 3. is only observed at high exposures not considered clinically relevant (see 2 above for exception) or 4. is similar to that induced by related agents, and the toxicity based on prior clinical experience with these related agents is considered a manageable risk If a study of reversibility is called for, it should be available to support clinical studies of a duration similar to those at which the adverse effects were seen non-clinically. However, a reversibility study is generally not warranted to support clinical trials of a duration equivalent to that at which the adverse effect was not observed non-clinically If a particular lesion is demonstrated to be reversible in a short duration (e.g., 2 week or 1 month) study, and does not progress in severity in longer term studies, repeating the reversibility assessment in longer term toxicity studies is generally not warranted If a reversibility study is warranted it is efficient to conduct it as part of a chronic study so that all toxicities of concern can be assessed in a single study provided that it is not critical to conduct it earlier to support a specific clinical trial

completed since January 2010 to reflect the revision and addendum of ICH M3 and ICH S6 guidelines. However, these revisions were not adopted by some regulatory authorities until after January 2010 and there were some other minor revisions to guidelines within the data collection period, including the publication of the ICH M3 Question and Answer document in March 2012. Data collection was completed in May 2013 (See Tables 3A and 3B).

For each individual study the questionnaire requested the rationale behind the decision to include or exclude recovery animals. The group devised a number of tick-box options to cover the most common rationale experienced by working group members. Respondents were permitted to tick multiple options and could use the open text field to provide additional information. Options included general reasons such as 'company practice' and 'perceived regulatory expectation' or more case-specific examples such as 'literature/known class effects', 'previous internal data on compound' or 'signal from prior *in vivo* study'. For each option ticked we asked whether this was a case-specific (related to the compound) or default reason (related to company practice).

For each study we asked for the impact that inclusion or exclusion of recovery animals had on both internal and regulatory decision-making. The group created appropriate tick-box options based on previous experience: impacts such as 'repeat study with inclusion of recovery', 'clinical hold' and 'modification of longer term studies' were included.

For the purpose of the questionnaire, the working group outlined a number of definitions. Recovery animal groups were defined as additional animals on the dosing groups that were retained for a pre-determined period of time (the recovery period) once treatment had been completed. Pivotal studies were defined as good laboratory practice (GLP) compliant toxicology studies. Partial recovery was defined as anything between no recovery and full recovery, and it was left to the respondents as to how this was interpreted. A default study design was defined as an "off the shelf" protocol, used historically by the organisation.

All studies included in the questionnaire had undergone approval through company ethical review and comply with national regulations (e.g. Animals (Scientific Procedures) Act 1986 (ASPA) and European Directives 86/609/EU or 2010/63/EU for studies carried out in the UK or EU, respectively). Companies provided data for between 5 and 12 compounds each. Completed questionnaires were provided in confidence by individual companies and data were collated and anonymized before analysis by the working group.

Analyses were separated for small molecules and biologicals, since a difference in approach to inclusion of recovery animals was expected due to the different scientific considerations for assessing safety between these drug classes.

3. Results

3.1. Overview of the dataset

Data on 137 compounds from 259 studies were collected from 22 pharmaceutical and biotechnology companies, including some submitted through CROs. For each compound, general information such as molecule type and therapy area was recorded (Fig. 1A and B). This was followed by more detailed and specific information on the individual studies carried out, such as the number of studies, species, dosing duration, inclusion of recovery animals and the number of recovery animals used (Tables 4 and 5 and Tables 8 and 9). Data were collected on the rationale for inclusion or exclusion of recovery animals and the impact this had on internal and regulatory decision making (Tables 6 and 7 and Tables 10 and 11).

The dataset included small molecules, 163 studies for 78 compounds, and biological compounds, 84 studies for 53 compounds. There was one synthetic peptide, and five compounds for which molecule type was given as 'other' (Fig. 1A). The data spanned a wide range of therapeutic areas, with oncology and neuroscience the most highly represented areas (Fig. 1B). Therapy area was recorded as 'other' for 17 compounds and was not given for 4 compounds.

Studies were conducted in rat, non-human primate (NHP) or dog (in 42.1%, 28.2% and 23.9% of studies, respectively), with a small number of studies carried out in other species, such as mouse (4.6%), rabbit (0.4%) or mini-pig (0.4%). The duration of the dosing period ranged from less than one week (single dose study) to a

Table 3A
A basic copy of the questionnaire (not including all tick box and selection options), showing the information requested for each study and compound. Participants were able to provide information on up to 10 studies per compound, for a maximum of 10 compounds. The complete questionnaire is included in Supplementary Table S1.

Compound information		
1	Type of molecule	[Select]
2	Therapeutic area	[Select]
3	Did the compound go into Phase I clinical trials?	[Select]
4	If yes, was Phase I in healthy volunteers or patients?	[Select]
5	What is the current status of this compound?	[Select]
6	If the compound was dropped, was this due to the non-clinical studies, business decision or other?	[Select]
7	Did data from recovery animals help the overall development programme?	[Select]

maximum of 26 weeks, with one to four studies carried out per compound.

The majority of compounds had recovery animals in all studies conducted to support FIH trials (101/137 compounds; 73.7%). A smaller proportion of compounds had recovery animals in 'some', but not all studies (11/137 compounds; 8.0%), or did not include recovery animals in any study (25/137 compounds; 18.2%) (Fig. 1C). The total number of recovery animals used ranged from 0 to over 100 animals per compound (Fig. 1D).

Data were separated into small molecules and biologicals for further analysis.

3.2. Small molecules

3.2.1. Small molecules – use of recovery animals

Recovery animals were included in all studies carried out for the majority of small molecules (51/78 compounds; 65.4%), though a significant proportion did not include recovery animals in any study (18/78 compounds; 23.1%) (Fig. 2A and Table 4).

Fig. 2B shows the number of studies carried out per compound and the number of studies that included recovery animals. Typically two studies were carried out per compound (57/78 compounds; 73.1%), one rodent and one non-rodent study, and recovery groups were usually included in both. Table 4 describes study designs for small molecules in more detail, looking at the number of studies carried out, the dosing duration, species, and inclusion of recovery animals. The most common approach was one rodent and one non-rodent study (typically rat plus dog or rat plus NHP), both 4 weeks in duration with recovery animals included in both studies (27/78 compounds; 35%).

Design of the individual studies with regards to inclusion of recovery animals were examined in more detail, to determine which dose groups typically included recovery animals (Fig. 2C and Table 5), as well as the size of the recovery groups (Table 5). Recovery animals were included in 111/163 small molecule studies (68.1%). For each approach, Table 5 also presents the total number of recovery animals used per study.

For each species, recovery animals were typically included in the control and one (usually high) dose group only (82/111 studies; 73.9%). Rat and dog were the most common species used, with fewer studies conducted in NHPs. Recovery group size varied depending on the species, with larger group sizes for rodents than non-rodents.

There were 82 studies in rats, 57 of which included recovery animals (69.5%). The most common approaches were inclusion of 5M + 5F per group in control plus high dose only, occurring in 19/57 (33.3%) or 10M + 10F per group in control plus high dose only, occurring in 15/57 studies (26.3%), and resulting in the use of 20 or 40 recovery animals per study, respectively. However, the maximum number of recovery animals used in any one study was 60 rats (10M + 10F in control plus two dose groups).

There were 53 studies in dogs, 35 of which included recovery animals (66.0%). The most common approach was recovery group sizes of 2M + 2F in control and high dose group only (25/35 studies; 71.4%). The total number of recovery animals per study ranged from 4 to 16 animals.

In total there were 25 studies in NHPs, 15 of which included recovery animals (60.0%). Recovery group sizes were always 2M + 2F. Recovery animals were either included in control plus one (10/15 studies; 66.7%) or control plus three dose groups (5/15 studies; 33.3%), resulting in the use of 8 or 16 recovery animals per study, respectively.

There were 5 studies in mice, 2 of which included recovery animals (40.0%). Recovery group sizes for these studies were 0M + 10F (i.e. females only) in control plus high dose group or 5M + 5F in control plus four dose groups, resulting in the use of 20 or 50 recovery animals per study, respectively.

Table 5 presents the number of recovery animals *per study* for the different approaches used. However, the total numbers of recovery animals *per compound* for small molecules ranged from 0 to 88. It should be highlighted here that there were 18 compounds that did not have recovery animals in any study.

3.2.2. Small molecules – rationale

When asked for the rationale behind the decision to include or exclude recovery animals, respondents were permitted to choose multiple rationales. Company practice was the most common reason for inclusion of recovery animals: for compounds where recovery animals were included (60/78 compounds), company practice was given as a rationale for inclusion for 41/60 (68%) compounds. Table 6 shows the complete list of rationales given for inclusion of recovery animals. 'Signal from prior *in vivo* study' ranked second (26/60 compounds; 43%) and 'perceived regulatory expectation' was also amongst the top ranked rationales (12/60 compound; 20%). 'Formal regulatory request' was rarely given as a reason for inclusion (only 1/60 compounds; 2%).

There were 18 small molecules that did not include recovery in any study prior to FIH. The top reason for exclusion of recovery animals was company practice, suggesting that for some companies it is their practice not to include recovery animals. Other common reasons given included 'lack of signal from prior *in vivo* study', 'previous internal data' and 'no indication of need'. The rationales for exclusion of recovery animals are given in Table 7.

3.2.3. Small molecules – impact

The impacts given for studies where recovery animals were included are shown in Table 6. The top impact reported was 'none', accounting for 27/60 small molecules (45%). However, this question was left unanswered or the impact was 'unknown' in a large proportion of cases. Specific impacts such as 'modification of the clinical programme/longer term studies' or 'additional studies' were less common.

It is important to note that multiple impacts could be chosen per compound/study. Compounds where a specific impact was reported (i.e. not including those described as 'none', or 'unknown') accounted for 16/60 (27%) compounds. These included impacts

Table 3B
A basic copy of the questionnaire (not including all tick box and selection options), showing the information requested for each study.

Study information Information on main study							
Species	How long was the dosing period?		How many animals were included per group in the main study?		How many dose groups were included in the main study?	Were recovery animals included?	If 'Yes' and recovery animals were included, what was the rationale for inclusion? *Please tick all that apply.* *Please indicate if this is default company practice?*
	Number	Unit	Male	Female			
[Select]	[Enter]	[Select]	[Enter]	[Enter]	[Enter]	[Select]	

Information on inclusion of recovery animals										
If 'Yes' and recovery animals were included, what was the rationale for inclusion? *Please tick all that apply.* *Please indicate if this is default company practice?*	How long was the recovery period?		What was the rationale for the duration of the recovery period? *Please tick all that apply.*	Were recovery animals included in the control group?	How many dose groups were recovery animals included in?	How many animals were included in recovery groups?		What was the outcome of the recovery?		What impact did inclusion of recovery animals have on the regulatory filing/clinical programme? *Please tick all that apply.*
	Number	Unit				Male	Female	What was the extent of recovery?	Was this expected or unexpected?	
Tick box options were provided here	[Enter]	[Select]		[Select]	[Enter]	[Enter]	[Enter]	[Select]	[Select]	Tick box options were provided here

Information on exclusion of recovery animals		
If recovery animals were NOT included, what was the rationale for exclusion? *Please tick all that apply.* *Please indicate if this is default company practice?*	What was the impact of NOT including recovery animals? *Please tick all that apply.*	Additional comments
Tick box options were provided here	Tick box options were provided here	Free text option

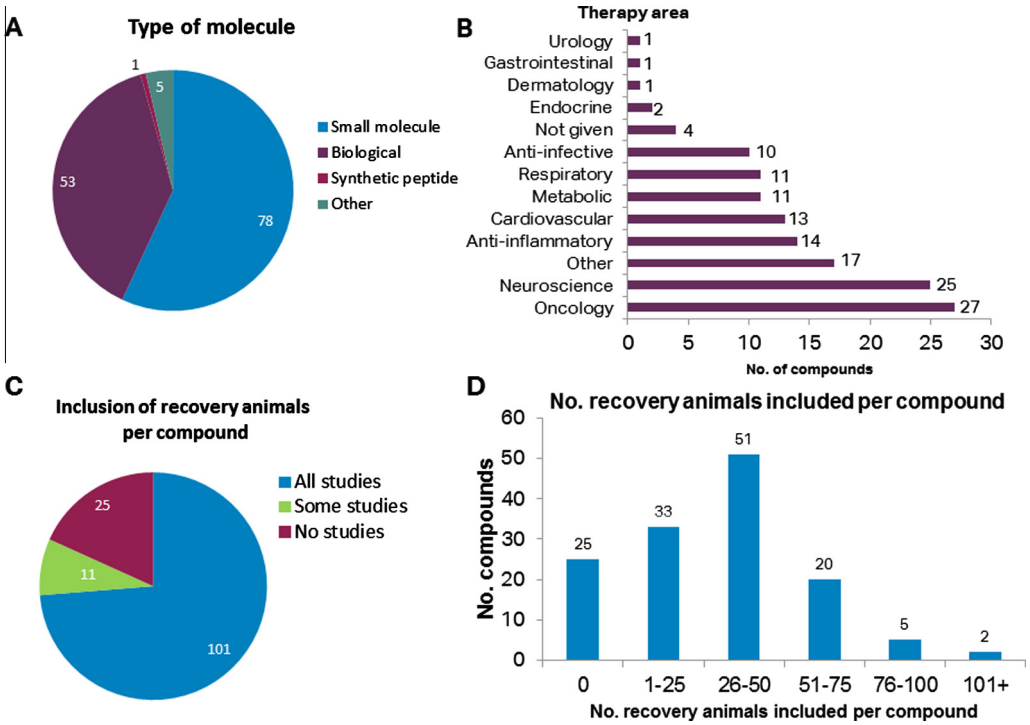

Fig. 1. Overall dataset (137 compounds from 259 studies) by molecule type (A), therapy area (B) and inclusion of recovery animals (C, D); inclusion of recovery animals in 'all', 'some' or 'no' studies carried out to support FIH per compound (C) and the total number of recovery animals that were included in these studies per compound (D).

reported under 'other' such as '*incomplete reversibility contributed to decision to terminate*' (1 compound) and '*reversibility of findings led to the removal of a previously set upper dose limit*' (1 compound).

For compounds that did not include recovery animals in any study (18 compounds) no impacts were reported (Table 7). All 18 of these compounds entered Phase I successfully without recovery data. At the time the questionnaire was completed in May 2013, 2 of these compounds had been discontinued, 10 remained in Phase I, and 5 compounds had progressed to Phase II or III. The status of the remaining compound was unknown.

For compounds where recovery animals were included in 'some', but not all studies, there were two cases where the impact of exclusion was 'a repeat study with recovery included' and one compound where the impact was 'unknown', as the compound had not yet gone into Phase I.

3.3. Biologicals

3.3.1. Biologicals – inclusion of recovery animals

Recovery animals were included in all studies for a large proportion of biologicals (45/53 compounds; 84.9%), though a small proportion did not include recovery animals in any study (6/53 compounds; 11.3%) (Fig. 3A and Table 8). It should be taken into consideration that for the majority of biologicals only one study was carried out, therefore 'all' for these compounds indicates inclusion on a single study, usually in the NHP.

Fig. 3B shows the number of studies carried out per compound and the inclusion of recovery animals. Typically one or two studies were carried out per compound, with recovery animals almost always included. Where more than one species was used, recovery was usually included in both rodent and non-rodent studies.

Table 8 describes approaches for biologicals in more detail, presenting details on the number of studies performed, the dosing duration, species, and inclusion of recovery animals. The most common approach was a single study in NHP of 4 or 13 weeks in duration including recovery animals, accounting for 19/53 compounds (36%). Typical approaches when two studies were performed were that there was one rodent and one non-rodent study (usually rat and NHP); both studies were also usually either 4 or 13 weeks in duration and almost always included recovery animals: compounds using this approach with recovery animals included in both studies accounted for 11/53 compounds (21%).

Design of the individual studies with regards to inclusion of recovery animals were examined in more detail, to determine which dose groups included recovery animals (Fig. 3C and Table 9) and the size of the recovery groups used (Table 9). Recovery animals were included in 69/84 studies (82.1%). For each approach, Table 9 also presents the total number of recovery animals used per study.

Recovery animals were always included in the control group, plus at least one and up to five dose groups, although inclusion in five dose groups was very rare (1 study). The most common approach was inclusion of recovery groups in control plus three dose groups (27/69 studies; 39.1%). There was a wide range in the size of the recovery groups used, dependent in part on the species used as outlined below.

In total there were 49 studies in NHPs, 44 of which included recovery animals. The most common approach used was inclusion of 2M + 2F recovery animals in the control plus one dose group (11/44 studies; 25.0%) or 2M + 2F in the control plus three dose groups (11/44 studies; 25.0%), accounting for 50% of all biological studies in NHPs and resulting in the use of either 8 or 16 recovery animals per study, respectively. However, given that the number of dose groups varied from one to five dose groups plus control, and recovery group sizes of up to 5M + 5F were used, the total number of recovery animals per study ranged from 8 to 50 NHPs.

Few dog studies were carried out for biologicals. Of the 6 dog studies in total only one included recovery animals; 2M + 2F recovery animals were included in the control and two dose groups, using 12 recovery animals in total.

Table 4
Small molecules: study design and inclusion of recovery animals in pivotal repeat dose toxicology studies to support first-in-human clinical trials, including the number of studies carried out per compound, the main study dosing durations, species used and whether recovery animals were included. The number of small molecule compounds in the dataset using each approach is indicated in the final column. The most common approaches are highlighted in bold.

Small molecules				
Number of studies	Dosing duration	Species	Inclusion of recovery animals	No. compounds
One study	4w	Rat	Yes	6
	4w	NHP	Yes	1
	13w	Mouse	No	1
(Total 8 compounds)				
Two studies	**4w + 4w**	**Rat + dog**	**Both**	**19**
			None	**8**
			One (rat)	**1**
	4w + 4w	**Rat + NHP**	**Both**	**8**
			One (rat)	**1**
	2w + 2w	Rat + dog	Both	4
			None	1
			One (Rat)	2
	2w + 2w	Rat + NHP	Both	1
			None	1
			One (NHP)	1
	2w + 4w	Rat + dog	Both	2
	4w + 4w	Rat + other	Both	2
	13w + 13w	Rat + dog	Both	1
		Rat + NHP	Both	1
	1w + 1w	Rat + dog	Both	1
	6w + 6w	Rat + NHP	Both	1
	8w + 8w	Rat + dog	None	1
	4w + 7w	Rat + NHP	One (7w; NHP)	1
(Total 57 compounds)				
Three studies	2w + 13w + 13w	Rat + rat + NHP	None	3
		Rat + rat + dog	None	2
	4w + 4w + 4w	Rat + dog + dog	All	2
		Rat + rat + dog	All	1
		Mouse + dog + dog	One (dog)	1
	2w + 2w + 2w	Rat + rat + dog	None	1
	4w + 4w + 13w	Rat + NHP + NHP	All	1
(Total 11 compounds)				
Four studies	2w + 2w + 2w + 13w	Dog + mouse + mouse + mouse	Two (2w mouse, 13w mouse)	1
	4w + 4w + 4w + 4w	Rat + rat + dog + dog	All	1
(Total 2 compounds)				

NHP = non-human primate; w = week.

There were 21 rat studies carried out for biologicals, 17 of which included recovery animals. There was no clear common approach for study designs using this species. The number of recovery animals per group ranged from 3M + 3F (1 study) up to 10M + 10F (4 studies), with 5M + 5F per group being the most common (7 studies). With varying animal numbers per group and inclusion in control plus one to four dose groups, the number of recovery animals included ranged from 20 to 100 rats per study.

There were 7 studies in mice, 6 of which included recovery animals. However, there was no common approach for mouse with regards to the use of recovery animals. Recovery group sizes ranged from 6M + 6F to 12M + 12F per group and the number of dose groups recovery animals were included on varied between control plus one and control plus three dose groups.

Table 9 presents the number of recovery animals *per study* for the different approaches used. However, the total numbers of recovery animals *per compound* for biologicals ranged from 0 up to a maximum of 170 animals. There were 6 compounds for which recovery animals were not included in any study.

3.3.2. Biologicals – rationale

When asked for the rationale behind the decision to include or exclude recovery animals, respondents were permitted to choose multiple options. For biologicals company practice and/or perceived regulatory requirement were the most common reasons for inclusion of recovery animals: recovery animals were included in 47/53 biological compounds, company practice and/or regulatory requirement was given as a rationale for inclusion in 34/47 (72.3%) of these. 'Biotherapeutic' was also one of the top reasons for inclusion of recovery animals, given for 24/47 (51%) biologicals. Table 10 shows the complete list of rationales given for inclusion of recovery animals for biologicals. More specific reasons included 'previous internal data', 'literature/known class effects' or 'perceived first in class'. Rarely, 'clinical request' or 'formal regulatory request', were given as reasons for inclusion.

There were 6 biologicals that did not include recovery animals in any study. The top reasons for exclusion were 'company practice' and 'lack of signal from prior *in vivo* study' suggesting that for some companies it is their practice not to include recovery animals, or that recovery animals are only included if there is an indication of need. The complete list of rationales for exclusion is shown in Table 11.

3.3.3. Biologicals – impact

The impacts reported for studies which included recovery animals are shown in Table 10. The top impact reported was 'none', accounting for 31/47 biologicals (66%) that included recovery animals. Impact 'unknown' was reported in a small proportion of cases (5/47 compounds; 10%). This was often accompanied with the additional text such as '*we cannot assess the impact as we don't know what would have happened if the recovery animals had been excluded*'.

Table 5
Small molecules: inclusion of recovery animals within individual studies, including the recovery group size, the number of dose groups that included recovery animals and the total number of recovery animals used per study. The number of studies for small molecules included in the dataset which used each approach is indicated in the final column. The most common approaches for each species are highlighted in bold. Please note there was also one study in mini-pig and one study where the species was not specified. There was one dog study where the numbers of recovery animals per group and the number of dose groups were not specified.

Small molecules				
Species	No. recovery animals per group	No. dose groups	No. recovery animals used	No. studies
NHP	**2M + 2F (15 studies)**	**Con + 1**	**8**	**10**
		Con + 3	16	5
(Total 15 studies)				
Dog	0M + 2F (1 study)	Con + 1	4	1
	2M + 2F (31 studies)	**Con + 1**	**8**	**25**
		Con + 2	12	3
		Con + 3	16	3
	3M + 3F (1 study)	Con + 2	12	1
	4M + 4F (1 study)	Con + 1	16	1
(Total 35 studies; details not specified for one dog study)				
Rat	3M + 3F (1 study)	Con + 1	12	1
	4M + 4F (4 studies)	Con + 3	32	4
	5M + 5F (27 studies)	**Con + 1**	**20**	**19**
		Con + 2	30	1
		Con + 3	40	7
	6M + 6F (9 studies)	Con + 1	24	8
		Con + 3	48	1
	10M + 10F (16 studies)	**Con + 1**	**40**	**15**
		Con + 2	60	1
(Total 57 studies)				
Mouse	5M + 5F	Con + 4	50	1
	0M + 10F	Con + 1	20	1
(Total 2 studies)				

NHP = non-human primate; M = male; F = female; Con = control.

Table 6
Small molecules: inclusion of recovery animals – rationale and impact. Recovery animals were included in 60/78 small molecules (111/163 studies). Please note that for each study, respondents were able to choose multiple rationales or impacts.

Small molecules						
Ranking	Rationale for inclusion	No. studies		No. compounds		No. companies
1	**Company practice**	**76**	(68%)	**41**	(68%)	11
2	**Signal from prior *in vivo* study**	**39**	(35%)	**26**	(43%)	10
3	Perceived regulatory expectation	24	(22%)	12	(20%)	6
4	Previous internal data on the compound	24	(22%)	15	(25%)	8
5	Oncology drug	24	(22%)	11	(18%)	4
6	Perceived first in class	22	(20%)	11	(18%)	5
7	Literature/known class effects	18	(16%)	10	(17%)	6
8	Clinical request (internal or external)	8	(7%)	5	(8%)	4
9	Healthy *vs.* patient population	4	(4%)	3	(5%)	3
10	Formal regulatory feedback	2	(2%)	1	(2%)	1
11	Unknown	1	(1%)	1	(2%)	1
Ranking	Impact of inclusion	No. studies		No. compounds		No. companies
1	**None**	**41**	(37%)	**27**	(45%)	**11**
2	Unanswered	18	(16%)	15	(25%)	7
3	Unknown	15	(14%)	6	(10%)	4
4	Modification of clinical programme	14	(13%)	8	(13%)	6
5	Other	11	(10%)	7	(12%)	4
6	Additional clinical studies	5	(5%)	4	(7%)	3
7	Modification of longer term studies	5	(5%)	4	(7%)	4
8	Internal delay	5	(5%)	4	(7%)	3
9	Formal regulatory request	3	(3%)	3	(5%)	3
10	Clinical hold	2	(2%)	2	(3%)	2

Specific impacts such as 'modification of clinical programme', 'additional clinical studies' and/or 'modification of longer term studies' were reported in a smaller proportion of studies. Often, multiple impacts were given per compound/study. Overall, there were only 8 compounds for which a specific impact(s) (i.e. other than 'none' or 'unknown') was reported. This included a single compound with an impact described under 'other' in which lack of reversibility '*contributed to termination of the compound*'.

For compounds that did not include recovery animals in any study (6 compounds) no impacts were reported (Table 11). Of these 6 compounds, 2 were discontinued prior to Phase I (for reasons unrelated to recovery) and 4 successfully entered Phase I. At the time the questionnaire was completed (May 2013) one of these compounds had been subsequently discontinued (for reasons unrelated to recovery) and the other three remained in Phase I.

Table 7
Small molecules: exclusion of recovery animals – rationale and impact. Recovery animals were not included in any study for 18/78 compounds (41 studies). This table lists the rationales for not including recovery animals and the impact that this had on internal and regulatory decision-making. Please note that for each study, respondents were able to choose multiple rationales or impacts.

Small molecules						
Ranking	Rationale for exclusion	No. studies		No. compounds		No. companies
1	**Company practice**	**28**	**(68%)**	**11**	**(61%)**	**3**
2	**Signal (or lack thereof) from prior *in vivo* study**	**26**	**(63%)**	**10**	**(56%)**	**3**
3	Previous internal data on the compound	26	(63%)	10	(56%)	2
4	No indication of need	14	(34%)	8	(44%)	5
5	Literature/known class effects (MOA)	4	(10%)	2	(11%)	2
6	Other	1	(2%)	1	(6%)	1
Ranking	Impact of exclusion	No. studies		No. compounds		No. companies
1	**None – other**	**41**	(100%)	**18**	(100%)	**6**

Table 8
Biologicals: study design and inclusion of recovery animals in pivotal repeat dose toxicology studies to support first-in-human clinical trials, including the number of studies carried out per compound, the main study dosing durations, species used and whether recovery animals were included. The number of biological compounds in the dataset using each approach is indicated in the final column. The most common approaches are highlighted in bold.

Biologicals				
Number of studies	Dosing duration	Species	Inclusion of recovery animals	Number of compounds
One study	**4w**	**NHP**	**Yes**	**10**
			No	**1**
	13w	**NHP**	**Yes**	**9**
	26w	NHP	Yes	2
	20w	NHP	Yes	1
	2w	NHP	No	1
	8w	NHP	Yes	1
	5w	Rabbit	Yes	1
	13w	Mouse	Yes	1
(Total 27 compounds)				
Two studies	**13w + 13w**	**Rat + NHP**	**Both**	**5**
	4w + 4w	**Rat + NHP**	**Both**	**4**
			None	**1**
	8w + 8w	Rat + NHP	Both	2
			None	1
	4w + 4w	Mouse + NHP	Both	1
			One (NHP)	1
	4w + 13w	Rat + NHP Mouse + NHP	Both	2
	5w + 13w	Mouse + NHP	Both	1
	8w + 8w	Rat + dog	Both	1
	13w + 13w	Mouse + NHP	Both	1
	13w + 26w	NHP + mouse	Both	1
	1w + 4w	NHP + Rat	Both	1
(Total 22 compounds)				
Three studies	4w + 4w + 4w	Rat + dog + dog	None	1
	6w + 6w + 6w	Rat + NHP + NHP	Two (rat, NHP)	1
	4w + 13w + 13w	Rat + rat + NHP	All	1
(Total 3 compounds)				
Four studies (Total 1 compound)	2w + 4w + 4w + 4w	Dog + rat + dog + dog	None	1

NHP = non-human primate; w = week.

There were also no impacts reported for exclusion of recovery animals for compounds where recovery was only included in 'some' studies (2 compounds).

4. Discussion

4.1. Current regulatory environment

The purpose of the questionnaire was to analyse current practices and examine the range of study designs used for assessment of reversibility of toxicity, as well as the impact this had on internal or regulatory decisions (from the pharmaceutical company perspective). Our data show that a variety of different study designs and approaches are used across the industry. This variation in company practice offers a possibility for making recommendations to reduce animal numbers, while taking into consideration international regulatory guidances.

Currently, it is a regulatory expectation that recovery from any toxic effects of a compound will be assessed at some point during the drug development process. However, there is flexibility in how this may be carried out. Prediction of recovery based on scientific assessment, e.g. through literature/known class effects, prior knowledge of the compound, or information gained from previous *in vivo* studies, is encouraged. Specifically, the current regulatory guidelines relating to the use of recovery animals state that inclusion of recovery animals is not always

Table 9
Biologicals: inclusion of recovery animals within individual studies, including the recovery group size, the number of dose groups that included recovery animals and the total number of recovery animals used per study. The number of studies for biologicals included in the dataset which used each approach is indicated in the final column. The most common approaches for each species are highlighted in bold. Please note there was also one study in rabbit.

Biologicals				
Species	No. recovery animals per group	No. dose groups	No. recovery animals used	No. studies
NHPs	2M + 2F (29 studies)	Con + 1	8	11
		Con + 2	12	5
		Con + 3	16	11
		Con + 4	20	1
		Con + 5	24	1
	3M + 3F (13 studies)	Con + 1	12	4
		Con + 2	18	3
		Con + 3	24	3
		Con + 4	30	3
	4M + 4F (1 study)	Con + 3	32	1
	5M + 5F (1 study)	Con + 4	50	1
(Total 44 studies)				
Dog	2M + 2F (1 study)	Con + 2	12	1
(Total 1 study)				
Rat	3M + 3F (1 study)	Con + 4	30	1
	5M + 5F (7 studies)	Con + 1	20	1
		Con + 2	30	2
		Con + 3	40	4
	6M + 6F (4 studies)	Con + 1	24	2
		Con + 3	48	2
	9M + 9F (1 study)	Con + 3	72	1
	10M + 10F (4 studies)	Con + 1	40	1
		Con + 3	80	2
		Con + 4	100	1
(Total 17 studies)				
Mouse	6M + 6F	Con + 1	24	1
		Con + 3	48	1
	7M + 7F	Con + 3	56	1
	9M + 9F	Con + 2	54	1
	10M + 10F	Con + 1	40	1
	12M + 12F	Con + 3	96	1
(Total 6 studies)				
Please note there was also one study in rabbit which included recovery animals				

NHP = non-human primate; M = male; F = female; Con = control.

Table 10
Biologicals: inclusion of recovery animals – rationale and impact. Recovery animals were included in at least one study for 47/53 biological compounds (69/84 studies). This table lists the rationales for inclusion of recovery animals and the impact that this had on internal and regulatory decision-making. Please note that for each study, respondents were able to choose multiple rationales or impacts.

Biologicals						
Ranking	Rationale for inclusion	No. studies		No. compounds		No. companies
1	**Company practice**	**41**	**(59%)**	**31**	**(66%)**	**11**
2	**Perceived regulatory expectation**	**36**	**(52%)**	**25**	**(53%)**	**6**
3	**Biotherapeutic**	**34**	**(49%)**	**24**	**(51%)**	**10**
4	Literature/known class effects	18	(26%)	15	(32%)	6
5	Previous internal data on the compound	13	(19%)	11	(23%)	7
6	Perceived first in class	11	(16%)	7	(15%)	5
7	Signal from prior *in vivo* study	9	(13%)	6	(13%)	5
8	Oncology drug	7	(10%)	5	(11%)	4
9	Clinical request (internal or external)	2	(3%)	2	(4%)	2
11	Other	2	(3%)	1	(2%)	1
10	Formal regulatory feedback	1	(1%)	1	(2%)	1
Ranking	Impact of inclusion	No. studies		No. compounds		No. companies
1	**None**	**46**	**(67%)**	**34**	**(72%)**	**9**
2	Other	7	(10%)	5	(11%)	4
3	Unknown	6	(9%)	5	(11%)	5
4	Modification of longer term studies	5	(7%)	3	(6%)	3
5	Modification of clinical programme	3	(4%)	3	(6%)	3
6	Additional clinical studies	2	(3%)	2	(3%)	2
7	Internal delay	1	(1%)	1	(2%)	1
8	Formal regulatory request	1	(1%)	1	(2%)	1

Table 11
Biologicals: exclusion of recovery animals – rationale and impact. Recovery animals were not included in any study for 6 biological compounds (13 studies). This table lists the rationales for not including recovery animals and the impact that this had on internal and regulatory decision-making. Please note that for each study, respondents were able to choose multiple rationales or impacts.

Biologicals						
Ranking	Rationale for exclusion	No. studies		No. compounds		No. companies
1	**Company practice**	**11**	**(85%)**	**4**	**(67%)**	**3**
2	**Signal (or lack thereof) from prior *in vivo* study**	**10**	**(77%)**	**4**	**(67%)**	**3**
3	Previous internal data on the compound	9	(70%)	3	(50%)	2
4	No indication of need	9	(70%)	3	(50%)	2
5	Literature/known class effects (MOA)	8	(62%)	3	(50%)	1
6	Other	1	(8%)	1	(17%)	1
Ranking	Impact of exclusion	No. studies		No. compounds		No. companies
1	**None**	**13**	**(100%)**	**6**	**(100%)**	**4**

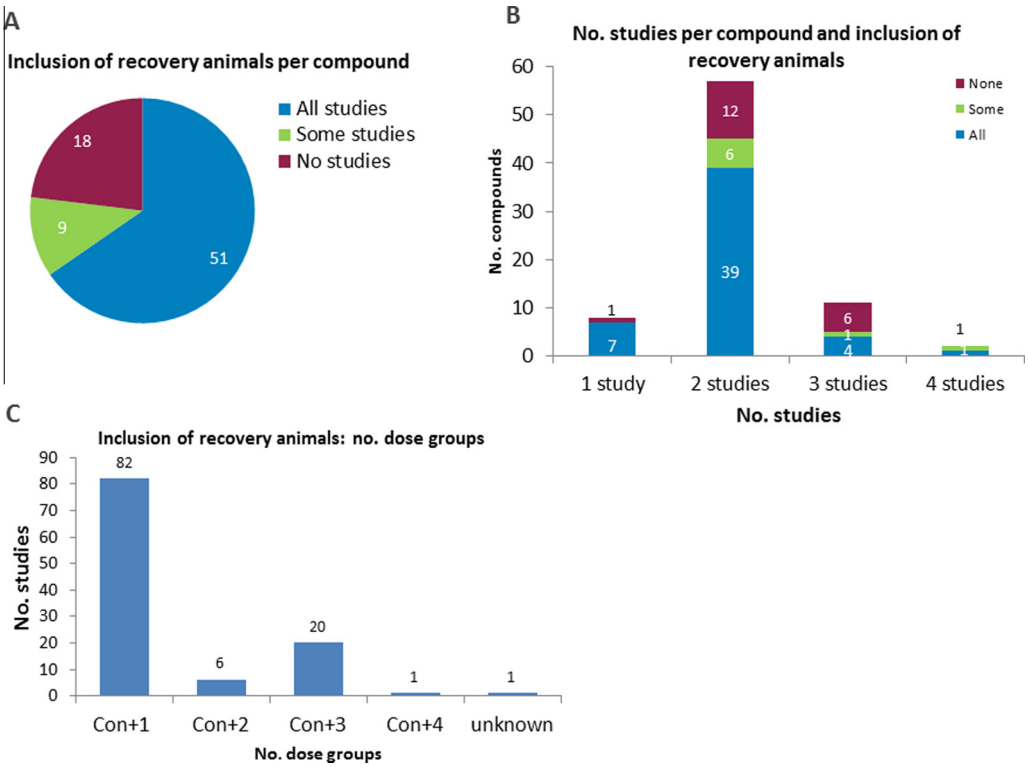

Fig. 2. Data on the inclusion of recovery animals for small molecule compounds (78 compounds from 163 studies); inclusion of recovery by inclusion of recovery animals in 'all', 'some' or 'no' studies carried out to support FIH per compound (A) and also by the total number of studies carried out per compound (B). For studies on which recovery animals were included (110 studies) the number of dose groups that they were included on are shown (C).

necessary, and that demonstration of complete reversibility of toxicity is not essential. Relevant extracts from the regulatory guidelines on the use of recovery animals are provided in Tables 1 and 2. These guidelines clearly state that although assessment of reversibility of toxic effects should be considered, this does not necessarily need to be demonstrated through addition of recovery animals, except in particular circumstances. This paper aims to provide recommendations and industry experience to support the information provided in the regulatory guidelines, to encourage inclusion of recovery animals only if scientifically justified, and to help overcome perceived regulatory barriers.

4.2. A variety of approaches

In order to capture how recovery animals are currently being used to support FIH, studies for small molecules and biologicals were examined to look at (i) approaches used within a drug development programme (e.g. rodent *vs.* non-rodent), (ii) approaches used within a study (e.g. number of dose groups or number of animals per group), (iii) the rationale for including or excluding recovery animals and most importantly (iv) the impact that this had on internal or regulatory decision-making.

Data on 259 studies from 137 compounds (including 53 biologicals and 78 small molecules) were examined and used as an evidence-base to make recommendations on the inclusion of recovery animals in toxicology studies to best assess human safety, while reducing animal use.

It is important to note that this is a retrospective analysis. Specifically, it was not the intention to comment, with the benefit of hindsight, on how the studies should have been designed. Instead, the aim was to use the data as an evidence-base to examine current practices and formulate recommendations for the use of recovery animals in future studies.

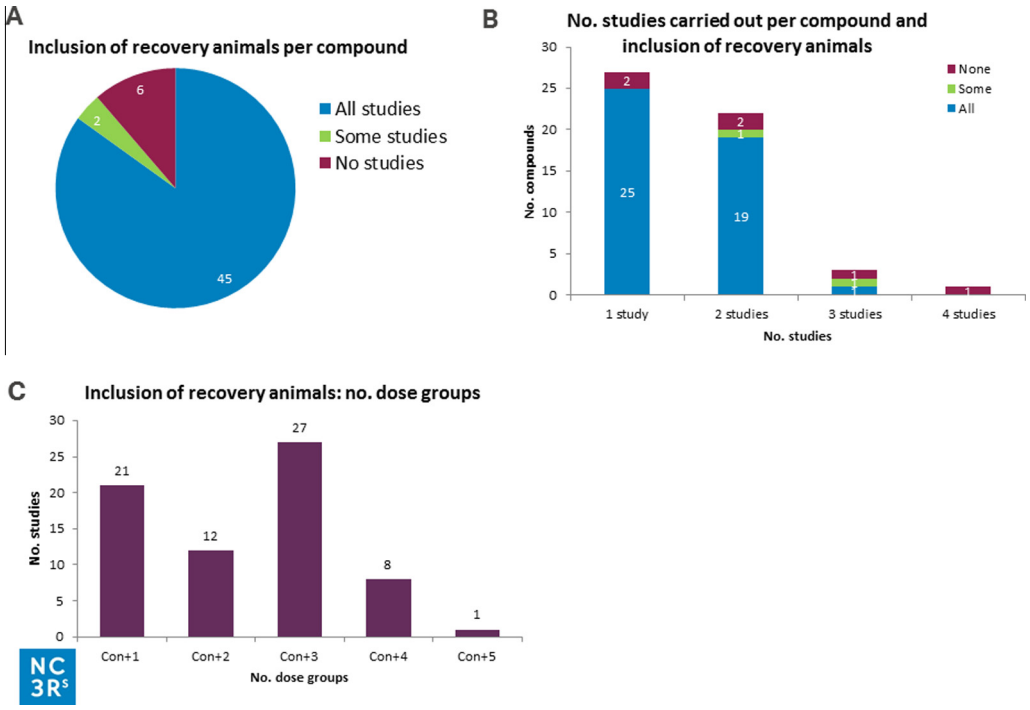

Fig. 3. Data on the inclusion of recovery animals for biological compounds (53 compounds from 84 studies); inclusion of recovery by inclusion of recovery animals in 'all', 'some' or 'no' studies carried out to support FIH per compound (A) and also by the total number of studies carried out per compound (B). For studies on which recovery animals were included (69 studies) the number of dose groups that they were included on are shown (C).

By using historical data, there were some limitations, as the required information was not always available or the answers given were ambiguous. This particularly applies to the more subjective questions of rationale and impact of inclusion/exclusion, which were more difficult to assess. Nevertheless, by looking at the most common answers given there was a clear indication of the top rationale and impacts for inclusion/exclusion. Limitations for the assessment of impact, particularly with regards to inclusion, are described in more detail in the section below.

It is important to note that participation in the survey was voluntary and so there was a potential for selection bias. However, an effort to minimise this bias was made by the selection criteria, where we requested that respondents provide information for compounds working backwards systematically, from the most recent compound back to January 2010.

More objective data were available regarding study design, such as information on the total number of repeat-dose toxicology studies carried out per compound, as well as data on the inclusion of recovery animals (i.e. number of dose groups and number of animals used per group), from 137 compounds and 22 companies, across a wide range of therapeutic areas, thus providing a clear picture of the use of recovery animals across the industry.

4.3. Rationale and impact for inclusion

Analysis of the dataset revealed a wide range in practice regarding the use of recovery animals for both biologicals and small molecules. Reasons for the different approaches may include risk aversion (e.g. inclusion of recovery animals in all studies to avoid the potential need to repeat studies and/or delay to the drug development or approval process), prior experience (e.g. the class of drug is known to cause toxicities that show evidence of reversibility) and/or use of different default study designs between companies.

Perceived regulatory expectation was one of the top rationales given for inclusion of recovery animals, despite the ICH guidelines stating that demonstration of reversibility of toxicity is only required in certain circumstances. There is a difference in response between small molecules (22%) and biologicals (52%) suggesting that regulatory expectation may be more of an issue for biologicals. The perceived regulatory expectations may reflect personal or company experiences with the regulators, a general sense of risk aversion to avoid unnecessary delays in drug development and a desire to progress to clinical trials in patients as rapidly as possible. For example, there appears to be a reluctance to alter default practices and omit recovery animals, as it is unknown how regulators will view the adequacy of the non-clinical safety characterisation and there remains a potential risk that companies could be asked to repeat studies to assess recovery (a fairly common reason given for inclusion of recovery animals was "*we don't know what would have happened if recovery animals had not been included*"). Default company practice was the other most common rationale for inclusion of recovery animals for both small molecules (68%) and biologicals (59%). For instance recovery animals were included in all repeat-dose studies and in all dose groups for 49% biologicals and 19% small molecules. However, it should be noted that only one repeat-dose study was conducted to support FIH trials for a significant number of biologicals (27/53 compounds; 50.1%) exaggerating the apparent difference in default company practices relative to small molecules. Regardless, the common use of a default approach for either type of compound represents an opportunity to reduce animal use.

Some compounds did not include recovery animals in any study: 6/53 (11%) biologicals and 18/78 (23%) small molecules. Only a small number of compounds (11/137: 8%) included recovery animals in 'some' studies. Within studies, recovery animals were usually included in the control and either one (typically the high dose) or all dose groups. For each compound, the same approach was used for each repeat-dose study that included recovery animals, suggesting that data from one study were not necessarily being taken into consideration in the design of subsequent studies.

Table 12
Recommendations. Recovery animals should not be included in general regulatory toxicology unless there is an indication of need. Situations where assessment of recovery may be warranted are outlined below, as part of a weight-of-evidence assessment. For clarity, situations where inclusion of recovery animals is not warranted have also been included. If inclusion of recovery animals is scientifically justified please refer to Fig. 4 for considerations and recommendations for study design.

Rationale for inclusion of recovery animals in general regulatory toxicology studies. Recovery animals should not be included unless scientifically justified. Where recovery animals are deemed a necessary inclusion, it is recommended that this occurs later in development, once more information is available to inform decisions and study design.
Weight-of-evidence pointing towards a need to include recovery animals: • Lack of prior knowledge: - lack of knowledge of the reversibility of specific lesion/effect e.g. not a common lesion - Lack of certainty of the MOA • Literature/known class effects: - indication of potentially irreversible (severe) toxicities at clinically relevant exposures (⩽10-fold clinical exposure) • Previous internal non-clinical data on the compound or a related one: - signal from prior *in vitro* or *in silico* study indicates that more information on reversibility is required - signal from prior *in vivo* study (e.g. from a prior study such as dose range finding) suggests reversibility may be a concern - suggestion of severe toxicity at clinically relevant exposures (e.g., ≤10-fold clinical exposure) • Prior internal clinical experience with class of drug: - suggests assessment of recovery is necessary (e.g. expected toxicities are not known to recoveror are expected to occur at clinically relevant levels) ICH, M3(R2) Q&A guidance • Severe toxicity at clinically relevant exposures (e.g., ⩽10-fold the clinical exposure) • Toxicity is only detectable at an advanced stage of the pathophysiology in humans and where significant reduction in organ function is expected even at clinically irrelevant exposures (e.g. >10-fold exposure multiples.)
Situations when inclusion of recovery animals is not warranted: • Reversibility (or lack thereof) can be predicted through scientific assessment (e.g. prior knowledge of the compound or class of compound) through sources such as: - Literature or otherwise known class effects (e.g. prior knowledge of the compound or class of compound and target; lesion effect known to be reversible or irreversible) - Previous internal non-clinical data on the compound or a related one - Prior internal clinical experience with class of drug - Expert experience on the nature of the lesion and reference to literature and other sources (i.e. toxicity is always reversible or always irreversible) • Known toxicities only occur at clinically irrelevant doses ICH, M3(R2) Q&A guidance • Toxicity is similar to that induced by related agents, and the toxicity based on prior clinical experience with these related agents is considered a manageable risk • Toxicity is only observed at clinically irrelevant high exposures • Toxicity of concern is irrelevant to humans (e.g. rodent Harderian gland toxicity) • Toxicity can be readily monitored in humans at an early stage before the toxicity becomes severe

For information provided on the impact recovery data had on internal or regulatory decisions, the most common answer was 'none'. Although, it is clear in this situation (i.e. no impact) that data from recovery animals did not influence internal decision making, either positively (i.e. to allow the programme to move forward) or negatively (i.e. it did not hold up compound progression), we do not know whether the recovery data contributed to regulatory approval to proceed to human studies. However, these data do indicate that there was at least no request for additional recovery data from the regulators. This could be because (i) recovery had been adequately assessed and/or (ii) there were no toxicities for which assessment of recovery was deemed necessary. The second most common answer was that the impact of recovery data on internal or regulatory decision was 'unknown'. This was often accompanied with a statement such as '*was not discussed with regulatory agencies what might happen if recovery had not been included*', which may account for 'perceived regulatory expectation' being given as the rationale for inclusion in a significant proportion of cases. In studies where recovery animals were included there were only four cases where the impact given was a formal regulatory request for recovery data (3 small molecules and 1 biological). In some cases inclusion of recovery animals did impact on the development programme. Reasons included modification of the clinical programme, requests for longer-term studies and/or clinical hold. However these were less common. The challenge is to identify the studies/compounds prospectively for which inclusion of recovery animals would be useful, rather than including them in studies where demonstration of recovery is unnecessary (i.e. where there is nothing to recover from or where full recovery is expected). This may involve including recovery animals in later studies once it is known whether there is something to recover from.

4.4. Science-based consideration

Despite the common approach to include recovery animals in all studies, there is a precedent for science-based consideration. Some companies do not include recovery animals unless there are case-specific reasons (i.e. it is 'default' practice not to include recovery animals). There are examples of compounds where recovery animals were not included on any pivotal study prior to FIH (25 compounds from 8 companies; including 6 biologicals and 18 small molecules). Reasons given for exclusion included 'company practice', 'no indication of need' and 'lack of signal in prior *in vivo* studies'. The impact of exclusion of recovery animals for these compounds was reported as 'none'. Contrary to inclusion of recovery animals, this term (i.e. impact 'none') is less ambiguous when considering the impact of exclusion of recovery animals. At a regulatory level, we know the compounds that subsequently entered Phase I were able to do so in the absence of recovery data: specifically 22/25 compounds that did not include recovery in any study successfully entered Phase I. The 3 compounds that did not enter Phase I trials were discontinued earlier in development for reasons unrelated to recovery.

Similarly, in 2008, Baldrick reported that in an evaluation of 34 FIH preclinical programmes with small molecules, 25 (74%) of these successfully entered clinical development without the inclusion of recovery groups, in either the rodent or non-rodent toxicology studies. This differs from our dataset, where only 18/78 (24%) small molecules did not include recovery animals, but were still able to enter FIH. Baldrick examined Investigator's Brochures used to support FIH from a 10 year period (1997–2006), whereas our dataset was collected from studies completed since January 2010. The difference may reflect changes in the regulatory environment (e.g. the revision and addendum of ICH M3 and ICH S6 guidelines

or perceived regulatory expectation), and/or the current status of the pharmaceutical industry.

There was a small proportion of compounds that included recovery animals in some, but not all, studies. For these compounds, an impact of exclusion was rarely reported. There were only three cases where an impact was described; two cases where a repeat study was required and another where the impact was marked as 'unknown' as the compound had not yet gone into clinical trials at the time of the questionnaire. Therefore, of all the studies that excluded recovery animals (69/259 studies), which comprises studies from compounds that included recovery animals in 'some' or 'no' studies, an impact was only reported in 3/69 studies (4.3%). The impact was described as 'unknown' for one of these studies, whereas repeat studies with inclusion of recovery animals were required for the remaining two. This demonstrates that where inclusion of recovery animals was not thought to be necessary, based on existing information from previous studies with the compound or other sources, there is limited risk associated with exclusion: specifically, repeated studies to assess recovery are rarely required.

4.5. Scientific justifications – when to include recovery animals

One of the reasons for starting this inquiry was anecdotal evidence suggesting that the industry may be moving towards the inclusion of recovery animals in more studies, paired with the desire of the working group to maintain a scientifically driven case-by-case approach. The working group recommends that recovery animals should only be included in studies to support FIH if there is a positive indication of need. This decision can be made through robust scientific assessment, where recovery animals are only included in situations where the weight-of-evidence points to a need to assess reversibility from a toxic effect using animals.

The working group has used the results of this analysis and their own experience to develop a list of circumstances where inclusion of recovery animals might be justified (Table 12). For clarity, a list of scenarios when inclusion of recovery animals may not be warranted has also been developed. This is the consensus opinion of the working group, which includes representation from 30 organisations, including pharmaceutical and biotechnology companies, contract research organisations, academia and regulatory bodies. The table is intended for use as a guide to identify appropriate situations for the inclusion of recovery animals, to minimise animal use, costs and resources, whilst avoiding risk to drug development and human safety.

Information from earlier studies should be used to make case-specific decisions on whether data from recovery animals are necessary. This could include previous non-clinical data on the compound itself or a related one, or clinical experience with the class of drug. If information from earlier internal studies is not available, evidence from the literature or the known mechanism of action may indicate whether assessment of recovery animals is necessary at a given stage of drug development. If the existing information is adequate to assess reversibility of any potential finding then recovery animals do not need to be included.

In situations where there is a lack of knowledge surrounding the potential reversibility of the toxicity seen in previous studies, and/or the type of lesion that may occur, then inclusion of recovery animals may be warranted. However, in most cases, it may be appropriate to delay assessment of recovery until more information is available (i.e. in later studies when it is known whether there is any toxicity from which to recover or if a scientific assessment would be adequate). If recovery assessment were postponed until after the initial safety studies to support FIH, the information generated from these studies can be used to make an informed decision on whether inclusion of recovery animals in later studies

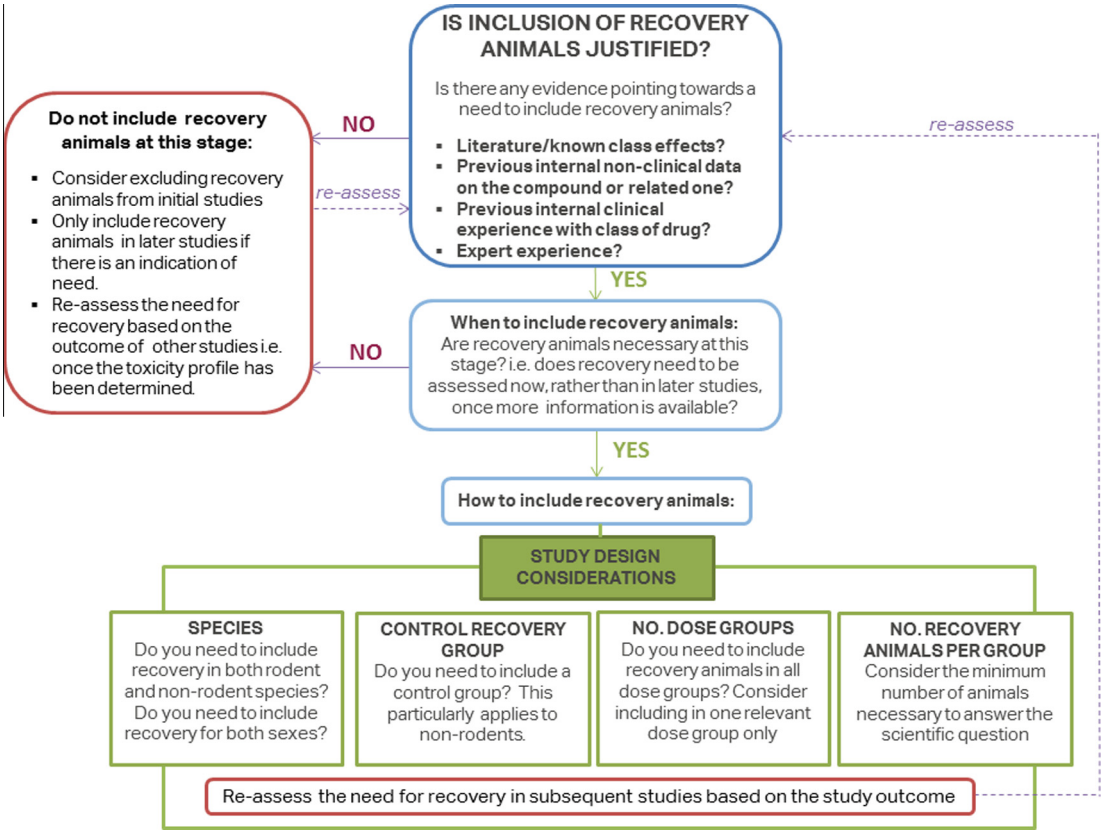

Fig. 4. A consideration tree to help inform decisions on when and how to include recovery animals in the development programme.

Table 13
All compounds: summary of the current approach to inclusion of recovery animals in studies conducted in mouse, rat, dog or NHP, using examples of the minimum and maximum approaches from the dataset (the number of dose groups recovery animals were included on, the number of recovery animals per group and the total number of recovery animals included per study).

Most common approach						
Species	No. dose groups with recovery animals		No. recovery animals per group		No. recovery animals per study	
Mouse	No common design		No common design		No common design	
Rat	Con + 1		5M + 5F		20	
Dog	Con + 1		2M + 2F		8	
NHP	Con + 1		2M + 2F		8	
Range in practice						
Species	No. dose groups with recovery animals		No. recovery animals per group		No. recovery animals per study	
	Min	Max	Min	Max	Min	Max
Mouse	Con + 1	Con + 4	5M + 5F	12M + 12F	20	96
Rat	Con + 1	Con + 4	3M + 3F	10M + 10F	12	100
Dog	Con + 1	Con + 3	2M + 2F	4M + 4F	8	18
NHP	Con + 1	Con + 5	2M + 2F	5M + 5F	8	50

is warranted, as well as to inform the appropriate design of those studies and reduce unnecessary animal use.

In view of the variability of the designs of preliminary *in vivo* toxicology studies, another consideration may be to include additional pathology (both histopathology and clinical pathology) into the initial non-clinical studies, in order to give some advanced warning on potential findings which could help in the design of later studies, including the decision on the need to assess reversibility. Although this may increase the financial cost of a preliminary study, it could potentially reduce the cost for the pivotal general toxicity study intended to support the FIH trial through reduced animal use. This may be particularly appropriate for small molecules. However, for biologicals where recovery from exaggerated pharmacology may need to be demonstrated the decision on when to include recovery animals during the development programme may be different.

4.6. Recommendations for study design

Recovery animals are included primarily to address one specific study objective, and that is to provide information on the reversibility of any treatment-related finding (though in rare cases they may identify delayed toxicity). If inclusion of recovery animals is scientifically justified, this can be performed using a well-considered study design. Accordingly, recovery animals do not necessarily need to be included in every study or on every dose group. The use of a study design that provides both adequate scientific information and an opportunity to reduce animal use is encouraged.

The expert industry working group have used their combined experience and expertise to give a series of considerations for study designs (Fig. 4). This is designed to be used in conjunction with Table 12, which provides a list of circumstances where inclusion of recovery animals may or may not be justified. Considerations include in which species to assess reversibility (rodent *vs.* non-rodent), the number of dose groups, and the number of animals per group, taking into account the range of different approaches utilised in this dataset (Table 13). There were large variations in the total number of animals used per study, depending on the number of dose groups that included recovery animals and the number of animals per group. For each species, Table 13 shows the most common approach used and the minimum and maximum approaches used.

In our dataset, when recovery animals were used they were generally included in one rodent and one non-rodent study. It should be a rare circumstance where recovery animals are needed in both rodent and non-rodent species. For example this may be appropriate if the toxicity is different in the different species and the reversibility for both species is unknown.

Recovery animals only need to be included at one appropriate dose level, rather than all dose levels. The dose level of choice is often the high dose group but there may be situations where the high dose is not the most appropriate. For biologics, for example, the high dose is not always clinically relevant, and inclusion in a lower dose group may be more appropriate.

A control recovery dose group may not always be necessary, particularly for non-rodents. In the absence of a control recovery group, the individual results for in-life evaluations at the end of the treatment period for each dose group can be compared with those at the end of the recovery period in the same animals to assess whether there has been a return to normal, so that each animal acts as its own control. For histopathology, reference can also be made to a historical control dataset (Konigsson, 2010). In the case of short recovery periods and with sexually mature animals, there is minimal risk of age-related phenotypic drifts, particularly in non-rodent studies. However, for studies with longer recovery periods and/or where juvenile or adolescent animals are used exclusion of the control recovery group may not always be appropriate. This especially applies to rodent studies, where the contribution of age-related phenotypic drifts may be more significant.

Generally, unless there are specific reasons for large group sizes (e.g. satellite groups for TK in rodents) minimal numbers of recovery animals should be used in each recovery group. The size of the recovery group used in any particular study may depend on the nature and prevalence of the expected toxicity. It is desirable to use the minimum number of animals required to achieve the answer to the scientific question. From the data obtained in the survey, the most common sizes for recovery animal groups were 2M + 2F for non-rodents and 5M + 5F for rodents.

Whether both male and female recovery groups are required should be driven by whether the lesion is seen in both sexes. Even so, if reversible in one gender, one would expect that this would reverse in both, and it may therefore be more appropriate to include adequate numbers in a single sex, rather than assess in both.

Overall, the data analysis shows that there is a precedent for science-based considerations regarding inclusion of recovery animals in repeat-dose toxicology studies. A common rationale for including or not including recovery animals in pivotal GLP studies was listed as being based on a signal (or lack thereof) from previous *in vivo* studies. This suggests that decisions on inclusion of recovery animals in pivotal studies may be effectively based on existing exploratory data or literature information in many cases. Therefore, consideration of such information should be encouraged in all cases as a means to reduce the number of animals used in recovery assessments. There were examples from our dataset that indicated minimal risk or impact when recovery animals were excluded from FIH studies in specific, case-by-case situations.

Compounds that did not include recovery animals in any study were able to successfully enter Phase I, in the absence of recovery data. The expert group has used the evidence-base and their combined expertise and experience to provide guidance on appropriate situations for the inclusion of recovery animals, to minimise animal use, costs and resources, whilst avoiding risk to drug development and human safety. By reviewing the impacts of inclusion, and providing examples of where exclusion has been appropriate, we can address the common misconception that inclusion of recovery animals is a universal regulatory requirement.

5. Conclusions

The dataset used for this assessment demonstrated a wide range of approaches to the inclusion of recovery animals to support FIH clinical studies, despite the common goal to meet global regulatory expectations. There are several examples where the absence of recovery animals had no impact on the regulatory processes. The approaches for small molecules and biologics were often different because of the nature of the materials.

The expert working group therefore has the following recommendations:

(1) Recovery phase animals are not included into any FIH non-clinical study design as default and should only be included for scientific reasons (Table 12).
(2) Inclusion of recovery phase animals should be considered across the whole development programme as well as individual studies to minimise animal use.
(3) The designs of the preliminary non-GLP studies should be carefully considered in order to obtain information so that an informed decision on the necessity to include recovery animals in the GLP-studies can be made.
(4) Consideration should be given to including recovery animals in later (rather than earlier) studies, where possible, once information on the toxicity profile is known.
(5) Consideration should be given to the most appropriate species (rodent *vs.* non-rodent) and sex in which to assess reversibility.
(6) The recovery group size should be kept to the minimum required to answer the scientific question, dependent on the nature and prevalence of the expected toxicity.
(7) The number of groups to which recovery phase animals are added should be kept to a minimum (e.g. control and one clinically relevant dose group only).
(8) For non-rodents, consideration should be given to not including recovery animals in the control group.

These recommendations, whilst not influencing the regulatory acceptability of any study, will result in a significant global reduction in the numbers of animals used to support FIH toxicity studies.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2014.07.018.

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