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Species selection for nonclinical safety assessment of drug candidates: Examples of current industry practice

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ABSTRACT

In drug development, nonclinical safety assessment is pivotal for human risk assessment and support of clinical development. Selecting the relevant/appropriate animal species for toxicity testing increases the likelihood of detecting potential effects in humans, and although recent regulatory guidelines state the need to justify or disqualify animal species for toxicity testing, individual companies have developed decision-processes most appropriate for their molecules, experience and 3Rs policies. These generally revolve around similarity of metabolic profiles between toxicology species/humans and relevant pharmacological activity in at least one species for New Chemical Entities (NCEs), whilst for large molecules (biologics) the key aspect is similarity/presence of the intended human target epitope.

To explore current industry practice, a questionnaire was developed to capture relevant information around process, documentation and tools/factors used for species selection. Collated results from 14 companies (Contract Research Organisations and pharmaceutical companies) are presented, along with some case-examples or over-riding principles from individual companies. As the process and justification of species selection is expected to be a topic for continued emphasis, this information could be adapted towards a harmonized approach or best practice for industry consideration.

Abbreviations: NCE, New Chemical Entity; MRF, Minipig Research Forum; CRO, Contract Research Organisation; FIH, First in Human; GTMP, Gene Therapy Medicinal Products; PK, Pharmacokinetics; PD, Pharmacodynamics; TK, Toxicokinetics; ADME, Absorption Distribution Metabolism and Excretion; NHP, Non-Human Primates; MABEL, Minimum Anticipated Biological Effect Level; MTD, Maximum Tolerated Dose; NOAEL or LOAEL, No or Low Observed Adverse Effect Level; BID, *Bis in die*; CSF, Cerebrospinal fluid; HA, Health Authority; $T_{1/2}$, Terminal elimination half-life.

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1. Introduction

In development of new medicines, nonclinical data is pivotal to support clinical studies at all stages and up to registration. Typically, a nonclinical package for safety evaluation encompasses *in silico*, *in vitro* and *in vivo* data, taking account of the various global and local country regulatory guidelines. A key part of the nonclinical data is assessment of potential safety risks for humans, usually including, but not limited to *in vivo* animal studies for general and reproductive toxicology, safety pharmacology, genetic toxicology, and carcinogenicity potential. The required nonclinical safety studies (and other research, such as primary and secondary pharmacology) can vary between molecule classes, but the basic format and principles are similar.

Overall, initial data from toxicology, safety pharmacology and pharmacokinetic (PK) studies provide safety and exposure information to support risk assessment and starting dose along with dose escalation scheme and any specific safety monitoring program for First in Human (FIH) clinical trials. These studies continue to cover safety prediction in terms of potential for toxicity through the clinical development period and form part of any marketing application process. This paper is focused on species selection for the general and reproductive toxicology studies, an important aspect of nonclinical safety assessment in the drug development process.

Regulatory guidance (Tables 1a and 1b) state that where possible two species, a rodent and non-rodent, are required for general and reproductive toxicology studies. For new chemical entities (NCEs, such as small molecules), at least one species should be "pharmacologically" relevant, i.e. the target expression, distribution and homology and the relative potency of the molecule against the target in the selected animal species and the intended patient population should be considered. The species should also be chosen based on their similarity to humans with

Table 1a
Summary of key points to be considered for species selection within various regulatory guidelines.[a].

Guideline	Summary of guidance – species choice or data to consider
ICH M3 (R2)	Minimal: *'two mammalian species (one non-rodent)'*.
ICH S1B	Minimal: *'in the absence of clear evidence favouring one species, it is recommended that the rat be selected'*.
ICH S2 (R1)	Minimal: *'both rats and mice are considered appropriate for use in the bone marrow micronucleus test'*.
ICH S5 (R3)	Section 5.1.1: *Selection of Species for DART Testing outlines general principles for selection of rat, mouse or rabbit.* Annex 1, Table 1: *outlines advantages and disadvantages to the use of various species utilized in DART studies.*
ICH S6 (R1)	Section 3.3 and Addendum 2.1*: outline general principles for use of relevant species and determination of relevance.*
ICH S7A	Minimal: '*Justification should be provided for the selection of the particular animal model or test system'*.
ICH S7B	Minimal: '*The most appropriate in vivo test systems and species should be selected and justified'*.
ICH S8	Minimal: *'be consistent, with the standard toxicity study in which an adverse immune effect was observed'*.
ICH S9	Minimal: '*usually includes rodents and non-rodents'*.
ICH S11	Section 3.3: *Animal test system selection outlines factors that should be considered when selecting a relevant species.*
EMEA/CHMP/SWP/ 28367/07 (R1)	Section 6.1: *describes appropriate tests to demonstrate relevance of animal models.*

Minimal: little information is described within the guideline – the specific wording is reproduced.
Where more information is available, the reader is referred to the specific section within the guideline. See Table 1b, the ICH website (ICH website, 2020) and EMEA, 2017 (EMEA, 2017) for further details.

[a] This list is not necessarily comprehensive for all guidelines which may be available globally, but focuses on those commonly used, internationally accepted and relevant for NCEs and biologics. Other guidelines are available within specific regions or for specific products such as vaccines, gene and cell therapies, advanced therapy medicinal products, etc. Checks for other or updated guidelines and policies is advised.

Table 1b
Relevant extracts from international regulatory guidelines regarding species selection and relevance for general toxicology studies[a].

ICH M3 (R2): Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals
No guidance on species choice, other than 'two mammalian species (one non-rodent)' in section 5.

ICH S1B: Testing for Carcinogenicity of Pharmaceuticals
4.2.1 Choice of species for a long-term carcinogenicity study. In the absence of clear evidence favouring one species, it is recommended that the rat be selected.

ICH S2 (R1): Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
4.1 Tests for the Detection of Chromosome Damage in vivo. Both rats and mice are considered appropriate for use in the bone marrow micronucleus test.

ICH S5 (R3): Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals
4.1.1 Strategy to Address Fertility and Early Embryonic Development (FEED). Such studies are typically performed in rodents. If the biopharmaceutical is pharmacologically active in rodents or rabbits, a FEED study in one of these species is recommended. Mating evaluations are not generally feasible in non-rodents such as dogs and NHPs.
4.2 Strategies to Address Embryo-Fetal Development (EFD). For most small molecules, effects on EFD are typically evaluated in two species (i.e., rodent and non-rodent (typically rabbit)). At least one of the test species should exhibit the desired pharmacodynamic response. The effect of biopharmaceuticals on EFD should typically be assessed in two species (one rodent and one non-rodent) if both are pharmacologically relevant. However, the rodent is often not pharmacologically relevant, in which case EFD assessment in a single pharmacologically relevant non-rodent species can be conducted. In cases where the NHP is the only relevant species, an enhanced pre-and postnatal development (ePPND) study can be conducted instead of an EFD study.
5.1.1. Selection of Species for DART Testing. The rat is generally appropriate for DART testing and is the most often used rodent species for reasons of practicality, general knowledge of pharmacology in this species, the extensive toxicology data usually available for interpretation of nonclinical observations and the large amount of historical background data. The mouse is also often used as the rodent species for many of the same reasons. For assessment of EFD only, a second mammalian non-rodent species is typically evaluated, although there are exceptions. The rabbit has proven to be useful in identifying human teratogens that have not been detected in rodents and is routinely used as the non-rodent species based on the extensive historical background data, availability of animals, and practicality.
5.1.2. Species Selection for Preventative and Therapeutic Vaccines. The animal species selected for testing of vaccines (with or without adjuvants) should demonstrate an immune response to the vaccine. The type of developmental toxicity study conducted, and the choice of the animal model, should be justified based on the immune response observed and the ability to administer an appropriate dose. Typically, rabbits, rats, or mice are used in developmental toxicity studies for vaccines. NHP should be used only if no other relevant animal species demonstrates an immune response.
Annex 1, Table 1: Principle Advantages and Disadvantages of Various Species for Developmental and Reproductive Toxicity Testing. Outlines advantages and disadvantages to the use of various species utilized in DART studies, with routine species (rat, rabbit, mouse) or non-routine species (NHP, mini-pig, hamster, dog).

ICH S6 (R1): Pre-clinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
3.1 General Principles. Preclinical safety testing should consider: 1) selection of the relevant animal species.
3.2 Biological Activity/Pharmacodynamics. Due to the species specificity of many biotechnology-derived pharmaceuticals, it is important to select relevant animal species for toxicity testing.
3.3 Animal Species/Model Selection. The biological activity together with species and/or tissue specificity of many biotechnology-derived pharmaceuticals often preclude standard toxicity testing designs in commonly used species (e.g., rats and dogs). Safety evaluation programs should include the use of relevant species. A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies). Relevant animal species for testing of monoclonal antibodies are those that express the desired epitope and demonstrate a similar tissue cross-reactivity profile as for human tissues. Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). Toxicity studies in non-relevant species may be misleading and are discouraged. When no relevant species exists, the use of relevant transgenic animals expressing the human receptor or the use of homologous proteins should be considered.
Addendum 2.1 General Principles. A number of factors should be taken into account when determining species relevancy. Comparisons of target sequence homology between species can be an appropriate starting point, followed by *in vitro*

(*continued on next page*)

Table 1b (*continued*)

assays to make qualitative and quantitative cross-species comparisons of relative target binding affinities and receptor/ligand occupancy and kinetics. Assessments of functional activity are also recommended.
Addendum 2.2 One or Two Species. If there are two pharmacologically relevant species for the clinical candidate (one rodent and one non-rodent), then both species should be used for short-term (up to 1 month duration) general toxicology studies.
Addendum 5.2 **Reproductive and Developmental Toxicity, Fertility.** For products where mice and rats are pharmacologically relevant species, an assessment of fertility can be made in one of these rodent species.
ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals
No guidance on species choice, other than 'Justification should be provided for the selection of the particular animal model or test system' in section *2.3.1.*
ICH S7B: The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
3.1 Considerations for Test Systems, in vivo Electrophysiology Studies. Laboratory animal species used for *in vivo* electrophysiology studies include dog, monkey, swine, rabbit, ferret, and guinea pig. The ionic mechanisms of repolarization in adult rats and mice differ from larger species, including humans (the primary ion currents controlling repolarization in adult rats and mice is I_{to}); therefore, use of these species is not considered appropriate. The most appropriate *in vivo* test systems and species should be selected and justified.
ICH S9: Nonclinical Evaluation for Anti-Cancer Pharmaceuticals
2.4 General Toxicology. For small molecules, the general toxicology testing usually includes rodents and non-rodents. In certain circumstances, determined case-by-case, alternative approaches can be appropriate (e.g., for genotoxic drugs targeting rapidly dividing cells, a repeat-dose toxicity study in one rodent species might be considered sufficient, provided the rodent is a relevant species).
Q&A Other considerations (applicable to ADCs). 4.10: Generally, two species are used for toxicology testing. For an ADC, are there situations where one species may be acceptable? When the antibody portion of an ADC binds only to human and NHP antigens, conducting a toxicity evaluation with the ADC in only the NHP (the only relevant species) would be appropriate, as discussed in ICH S6(R1).
ICH S11: Nonclinical safety testing in support of development of paediatric pharmaceuticals
3.3 Animal test system selection. When a JAS is warranted, in most cases a single species is considered sufficient. In principle, the same species as used in adult repeated-dose studies should initially be considered as the species for a JAS, preferably a rodent. In all cases, the selected species should be justified, as nonclinical studies in a pharmacologically non-relevant species can give rise to misinterpretation and are not recommended.
The following factors should be considered when selecting a relevant species: i) An understanding of the ontogeny of the pharmacological or toxicological target (e.g., the receptor) in animals in comparison to that in the intended paediatric population; ii) Preference for a species and strain for which adult repeated-dose toxicity data are available to facilitate a comparison of the toxicity and systemic exposure profiles between juvenile and adult animals; iii) Toxicological target organs; iv) Similarity to human ADME characteristics; v) The technical/practical feasibility to conduct the study in the selected species. Advantages and disadvantages of using different rodent (rat, mouse) or non-rodent (rabbit, dog, minipig, NHP) species are outlined in Appendix A, Table A6.
EMEA/CHMP/SWP/28367/07 Rev. 1: Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products
6.1 Demonstration of relevance of the animal model. The relevance of the selected animal model should be justified in the CT application. The demonstration of relevance of the animal model(s) may include comparison with humans of: i) target expression, distribution and primary structure. However, a high degree of homology does not necessarily imply comparable effects; ii) pharmacodynamics; iii) metabolism and other PK aspects; iv) on- and off-target binding affinities and receptor/ligand occupancy and kinetics.
For small molecule entities, in line with ICH M3 (R2), at least one species used for toxicity testing (rodent or non-rodent) should be "pharmacologically" relevant, where both the presence of the target and the relative potency of the molecule against the target in the selected animal species and the intended patient population should be considered. The species should also be chosen based on their similarity to humans with regard to *in vitro* metabolic profile.
For biotechnology-derived products, and in line with ICH S6 (R1), studies in non-relevant species may give rise to misinterpretation and are discouraged. Where no relevant species exists, the use of homologous proteins or the use of relevant transgenic or humanised animals expressing the human target should be considered.

[a] This list is not necessarily comprehensive for all guidelines which may be available globally, but focuses on those commonly used, internationally accepted and relevant for NCEs and biologics. Other guidelines are available within specific regions or for specific products such as vaccines, gene and cell therapies, advanced therapy medicinal products, etc. Checks for other or updated guidelines and policies is advised. See the ICH website (ICH website, 2020) and EMEA, 2017 (EMEA, 2017) for further details.

regards to *in vitro* metabolic profile, on- and off-target binding affinities and receptor/ligand occupancy and kinetics. The choice of species for biologics is also dictated by pharmacological relevance and single species toxicology programs (typically using the NHP) are often adequate (Brennan et al., 2018; Prior et al., 2020a). Information on how species are selected for general toxicity studies is typically sparse in the public domain. An IQ DruSafe consortium perspective highlights criteria for species selection that includes knowledge of species similarities or differences compared with humans, regarding target characterization (receptor expression, homology, distribution, subtypes), physiology/pharmacology, metabolism/PK (e.g. metabolite profile, plasma protein binding), and class related tolerance or safety precedents (Butler et al., 2017). More recently, justification for species selection were published from a cross-industry database, along with comprehensive insight into the decisions made within two major pharmaceutical companies (Prior et al., 2020b).

For species selection it is important to understand which studies/research are required, how this is documented, who is involved and how decisions are made. Appropriate species selection is expected to facilitate better translatability of nonclinical data to human. In addition, such information may assist in situations where a switch of species is required during development, which is often a challenging task. In general, the regulatory guidance for species selection is high-level and not detailed (Tables 1a and 1b) i.e. a flexible format to cover a variety of molecules. The approach for using two species (rodent and non-rodent) is outlined, but no information is provided as to which species/strain/breed of rodent or non-rodent should be utilized. Although use of 'standard' species has been adopted due to widespread experience and acceptance (e.g., typically (Han)Wistar or Sprague Dawley (SD) rats and predominately Beagle dogs for NCE development) and is well documented in the literature (Butler et al., 2017; Baldrick, 2017), there are alternative species that could be considered by Sponsors. The minipig as a non-rodent species, is perhaps a prime example of species not included routinely by many Sponsors (Jones et al., 2019) but the same can be said too for others e.g. marmoset and different strains of rodent. A key update regarding regulatory perspectives on the importance and relevance of species selection occurred in 2017, when the European Medicines Agency (EMA) revised its guidance on FIH clinical trials to help stakeholders identify and mitigate risks for trial participants (EMEA, 2017). It clearly states that the relevance of the selected animal model(s) needs to be justified in the clinical trial application.

In order to further explore current industry approaches to species selection for nonclinical safety studies, a 'Species Selection Interest Group', inspired by the Minipig Research Forum (MRF) was formed. As background, attendees of the MRF annual meetings in 2018 and 2019 participated in interactive workshops on 'species selection', to discuss use of the minipig within safety assessment packages and how/when the minipig was included within species selection decision-making (Jones et al., 2019). This led to a more general discussion of how rodent and non-rodent species are selected as the toxicology species within different companies. The group consists of experienced toxicologists and pathologists from various global pharmaceutical and biotechnology companies and 3 contract research organisations (CROs) and was led by independent members of the MRF Steering Committee. The overall aim of the group was to investigate the processes or guidelines individual companies followed for species selection, including the number of species/strains/breeds assessed and the specific tests/factors considered to justify the decisions.

This paper highlights aspects of species selection and focuses on:

1. Current regulatory requirements/expectations
2. The disciplines/groups in industry that are involved, the process, who makes decisions and how this is documented
3. The species/strains/breeds of rodent and non-rodent that are used "routinely" and why

4. The studies/data ("factors") that are considered and/or used to assist in species selection
5. Compare and contrast the approaches taken with NCES and biologics

A questionnaire devised to explore the key aspects of species selection as highlighted above, was sent to several biopharmaceutical/pharmaceutical (industry) companies and CROs to obtain data and the results were examined and discussed. Furthermore, we have provided some brief case examples of species selection approaches and the process/documentation employed.

As most new medicines in development fall into the categories of NCEs (small molecules or other modalities following ICH M3 (R2) guidelines (ICH M3(R2), 2009)) or biologics (large molecules following ICH S6 (R1) guidelines (ICH S6(R1)), this paper also provides comparative analysis of the approaches used for these classes of molecules. Other molecules/entities such as oligonucleotides, vaccines, gene therapy medicinal products (GTMPs), follow slightly different regulatory paths and/or regulatory guidance, but many aspects of species selection could be considered appropriate and applied to these molecules. These will be addressed in the paper where appropriate.

2. Materials and methods - questionnaire

2.1. Data collection

A Microsoft® Excel®-based questionnaire was developed by three members of the group, two independent to any company. Drop-down menus with pre-populated answers facilitated completion of the questionnaire, along with tick-box options for multiple answers and open comment boxes for free-text additional information where applicable. Following review and updates to the questionnaire by the whole author group, a period of 8 weeks was allowed for data collection (April–May 2020). The questionnaire was sent to various (bio)pharmaceutical companies and CROs, which included the co-author companies and other contacts known to the authors expressing an interest to participate. One completed questionnaire was requested for each company, with the contact person referring to other colleagues/sites, as needed. The companies were mainly based in the EU, but also included the USA, Canada, and Japan. This distribution was considered sufficient to provide a reasonable dataset and adequate insight of current practice since it included examples of big, mid-size and small (bio)pharmaceutical companies along with major CROs from various jurisdictions.

The questionnaire focussed on species selection for general and reproductive toxicology, as a major component of nonclinical safety studies, but there was no separation of questions or responses between these broad categories. Carcinogenicity and other nonclinical studies (such as *in vivo* safety pharmacology and genotoxicity) were not included within the remit, since species selection is either clearly specified within regulatory guidelines or generally follows the species selected for general toxicology studies. The following aspects were addressed, split into three main sections:

1) Process and documentation: General information on the functions/disciplines involved in the toxicology species selection process and the final decision maker(s); whether companies had a formal process to guide or outline the steps/data to consider for toxicology species selection, how this was documented and how the decision was recorded; questions relating to regulatory interactions to determine if information to justify the toxicology species used was included within regulatory submissions, and if the choice of toxicology species had been questioned/rejected or evaluation of additional species requested by regulatory authorities.
2) Species: The rodent and non-rodent species and strains/breeds that were typically considered (defined as 'thought about using information in literature and/or previous knowledge within project') or assessed (defined as 'tested this species for suitability'), and those which were considered on a case-by-case basis. In addition broad groups of NCEs and biologics were considered separately.
3) Factors: a comprehensive list of *in silico, in vitro* and *in vivo* tests or factors, grouped within categories of pharmacology, ADME/PK or toxicology (see Table 2), to document which tests/factors were always/frequently or rarely/never considered. As before, NCEs and biologics were considered separately.

2.2. Data analysis

Questionnaires were returned to one of the independent group members and blinded to avoid any bias or knowledge of individual company processes (Company A, B, C, etc). The data was collated (blinded) and QC-checked by the three members who had prepared the questionnaire, before sharing (blinded) for discussion by the whole group.

3. Results

3.1. Dataset demographics

Fourteen completed questionnaires were received from 11 (bio) pharmaceutical companies and 3 CROs from within the UK and Europe (8), Japan (4) and USA or Canada (2).

All of the companies provided data for NCEs (n = 14) but one company did not provide the detailed level of information requested for NCEs for the factors section, so results reported in Table 2 are based on n = 13. For biologics, one company did not work with these types of molecules/products and another company did not provide the detailed information for the factors' section, so results reported in Table 2 are based on n = 12. Additionally, for Table 2, some companies provided a response of not applicable (N/A) instead of one of the three available categories, indicating the specific test/factor was not applicable for either NCEs or biologics. However, since a response of N/A was considered a valid input it was included within the table.

3.2. Process and documentation

The functions/disciplines that contribute to the toxicology species selection process tend to be a collaboration of project team representatives from pharmacology, ADME/PK and toxicology disciplines (Fig. 1a), with the majority (12 of the 14 respondents) replying that the toxicology function has the over-riding decision.

Most of the organisations stated that they have formal or semi-formal processes to guide or outline the steps, or data to facilitate species selection (10/14 respondents). These processes included working practices that provided data requested on CRO pre-study approval forms, or templated slide-decks for milestone presentation meetings (Fig. 1b). Often, additional information was requested if specific species were being considered (Fig. 1c), or restrictions were in place for the use of some species. For example, one respondent reported they tried not to use marmosets due to a lack of background data; another reported that additional scientific rationale was required to use dog in cases where minipig or cynomolgus monkey was shown to be unsuitable, whilst others reported additional scientific rationale was required to use NHPs due to European animal welfare policies.

All organisations included information within regulatory submissions to justify the toxicology species used (Fig. 1d). Just over 60% (9/14 respondents) of the organisations had not received requests to provide additional information to justify the selected species, whilst others reported examples of the choice of toxicology species being questioned or rejected (36%, 5/14 respondents). No further information was provided to expand the rejection of species scenarios, whilst most of the questions or requests for more information pertained to pharmacological relevance for biologics and/or the use of one species for chronic toxicology studies with biologics. There were only a few requests to

Table 2
Tests and other factors considered during toxicology species selection – results from industry responses to the Questionnaire.

Factors	New Chemical Entities (NCEs)[a]				Biologics[b]			
	Always/ frequently consider	Rarely/ never consider	Not currently using, but should do in future	Not applicable (N/A)	Always/ Frequently consider	Rarely/ never consider	Not currently using, but should do in future	Not applicable (N/A)
in vitro considerations								
Pharmacology								
Target homology	85%	15%			100%			
Pathway/Mode-of-Action data	85%	8%	8%		92%	8%		
Expression levels of the target	69%	23%	8%		100%			
Binding affinity and kinetics	62%	38%			100%			
Functional activity and/ or potency	54%	31%	15%		83%	8%	8%	
Tissue distribution of target	54%	38%	8%		67%	33%		
Tissue cross-reactivity		69%		31%	42%	58%		
ADME/PK								
Plasma protein binding	77%	15%	8%		25%	42%		33%
Clearance	62%	31%	8%		42%	42%		17%
Predicted $t_{1/2}$	62%	31%	8%		50%	42%		8%
Metabolite profile	92%		8%					
Similarity of metabolite profile cross-species	92%		8%		25%	50%		25%
Tissue/Target organ to plasma ratio	15%	77%	8%		8%	75%		17%
Toxicology								
Cytotoxicity	31%	69%			25%	75%		
Species specific toxicity/ tolerability [c]	69%	23%	8%		42%	58%		
in vivo considerations								
Pharmacology								
Availability of disease models	31%	54%	15%		67%	25%		8%
Translatability of *in vivo* models	46%	46%	8%		75%	17%		8%
Ease of creating transgenic or knockout models	15%	62%	8%	15%	42%	42%	17%	
Efficacy model availability for indication expansion	23%	54%	15%	8%	8%	75%	8%	8%
Existence of biomarkers of disease	38%	54%	8%		42%	50%	8%	
Degree of PK/PD data correlation	77%	23%			75%	25%		
Ease of establishing MABEL	8%	69%	15%	8%	50%	50%		
Efficacy demonstrated in PD model	62%	23%	15%		67%	25%	8%	
ADME/PK								
Bioavailability	100%				75%	17%		8%
PK profile shape	69%	31%			58%	42%		
Dose proportionality	54%	46%			67%	33%		
Clearance and/or T1/2	77%	15%	8%		75%	25%		
Male & female PK difference	46%	46%	8%		33%	67%		
Tissue/Target organ to plasma ratio	23%	62%	15%		8%	92%		
Toxicology								
Suitability for the intended route of administration	100%				100%			
Need for alternative route of administration	38%	54%	8%		50%	33%	8%	8%
Sensitivity/tolerability and nature of toxicity	92%	8%			92%	8%		
	69%	23%	8%		83%	17%		

(*continued on next page*)

Table 2 (*continued*)

Factors	New Chemical Entities (NCEs)[a]				Biologics[b]			
	Always/ frequently consider	Rarely/ never consider	Not currently using, but should do in future	Not applicable (N/A)	Always/ Frequently consider	Rarely/ never consider	Not currently using, but should do in future	Not applicable (N/A)
Exposure coverage at MTD, LOAEL or NOAEL								
Relevance of toxicological findings to human	85%	15%			92%	8%		
Availability of safety pharmacology models	54%	38%	8%		33%	42%	8%	17%
Combining safety pharmacology into Tox	62%	31%	8%		83%	17%		
Availability of historical/ background data	92%	8%			92%	8%		
Anatomical and physiological relevance	92%		8%		92%	8%		
Potential for Juvenile toxicity evaluation	54%	46%			33%	67%		
Blood volume for additional samples	62%	38%			75%	25%		
Formulation/excipient tolerability	92%	8%			92%	8%		
Ease of BID administration	46%	46%	8%		17%	83%		
Test article requirements	69%	31%			73%	27%		
Ethical considerations (e. g., use of NHPs)	92%	8%			83%	17%		
In silico Considerations	23%	54%	23%		33%	67%		

Other considerations added into comments box: NCEs: 1. Shipment of monkey samples; 2. Minipig - not so often used due to limited ADME data in-house and higher amount of test item needed; 3. Cost of study for different species; amount of resource.

[a] All respondents worked with NCEs but one respondent did not provide answers to this question (n = 13).

[b] Thirteen respondents worked with biologics but one respondent did not provide answers to this question (n = 12).

[c] Species specific tissue toxicity/tolerability.

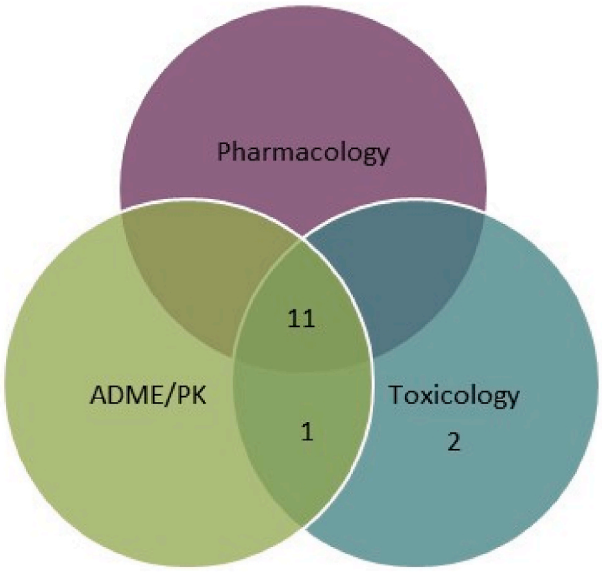

Fig. 1a. Which functions contribute to the toxicology species selection process? Respondents (n = 14) could select multiple answers and provide additional comments. The Venn diagram represents the interactions within multiple main disciplines involved in these decisions. One respondent indicated additional input from bioinformatics team which is not shown in the diagram.

perform additional studies in another/different species, again related to chronic toxicology studies with biologics (use of two rather than one species), or additional species to investigate specific toxicological findings.

3.3. Species considered for nonclinical safety assessment of NCEs

All organisations typically considered the rat as the primary NCE rodent species, whilst most also considered the mouse (Fig. 2a) as an alternative. Other rodent species (e.g. guinea-pig, hamster) were considered on a case-by-case basis. There was a greater variety of combinations of non-rodents typically considered, with 3 organisations considering only one non-rodent species (either dog or NHP) and others considering either two (3 organisations) or three non-rodent species (7 organisations). The dog, NHP, minipig and rabbit were all non-rodent species considered on a case-by-case basis (except for reproductive toxicology studies, since rabbit is generally the default second species for embryofetal toxicity studies). Among these species the minipig was most often considered (e.g., 45%) (Fig. 2b).

The rodent strains most commonly considered were outbred (Han) Wistar and/or SD rat and CD-1 mouse, with a range of other strains considered case-by-case basis (Fig. 2c). The non-rodent breeds most considered were beagle dog, cynomolgus monkey and Göttingen minipigs with a range of other breeds considered on a case-by-case basis.

3.4. Species considered for nonclinical safety assessment of biologics

Most organisations considered both the rat and mouse for the testing of biologics (10 organisations), although 3 organisations considered only one of these rodent species (Fig. 3a). Other rodent species were only rarely considered. All organisations considered the NHP as the non-rodent species of choice for biologics, either as the only species considered (7 organisations) or in combination with dog and/or minipig and/or rabbit (Fig. 3b).

The rodent strains most considered were outbred (Han)Wistar and/or SD rat and CD-1 mouse, with other strains considered as required (Fig. 3c). The non-rodent breeds most considered were beagle dog, cynomolgus monkey, Göttingen minipigs and New Zealand white rabbit,

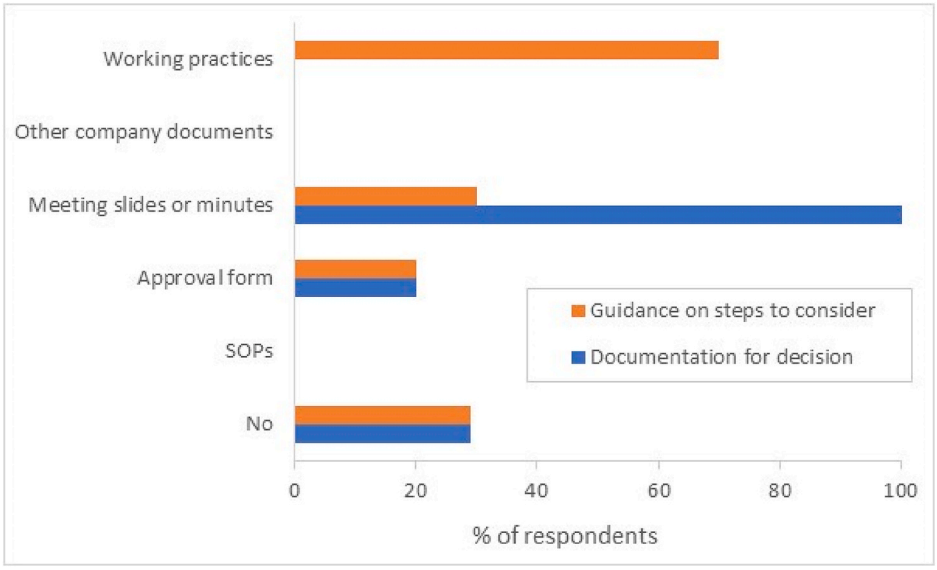

Fig. 1b. Are there formal practices to guide species selection and document the decision? The % of respondents with formal process (es) to guide species selection and to document the decision. Questions were: 'Do you have a formal process to guide or outline the steps/data to consider for toxicology species selection?' and 'Do you have a formal process for documenting the decision(s) taken for toxicology species selection?'. If No (4 respondents to both questions) this is presented as % of total respondents (n = 14). For the respondents answering Yes (10 respondents to both questions) further information 'How is this documented?' was requested, where multiple answers could be selected, or free-text comments provided. These were collated into similar categories and presented as % of positive respondents (n = 10).

Fig. 1c. Are there different questions/considerations for specific species? Respondents (n = 13) indicated a Yes or No response, and most provided additional information via free-text comments. These were collated into similar categories and presented in the right-hand pie-chart above. Examples are included in the results text. Numbers within the chart gives the actual number of respondents, for ease of reading.

with other breeds considered as required.

3.5. Tests/factors considered for NCEs and biologics toxicology species selection

The data summary and presentation of the results for factors used and considered to assist with species selection for NCEs and biologics outlined in Table 2 are generally based on the proportion (%) of respondents (companies). For ease of comparison, results for both groups of molecules are shown side by side (Table 2) but for clarity are discussed separately. Factors were divided based on model system/study type i.e. *in silico*, *in vitro*, *in vivo* research, with the latter two sub-divided into functional research areas "Pharmacology", "ADME/PK" (Absorption, distribution, metabolism, excretion/pharmacokinetics) and "Toxicology".

3.5.1. NCEs

3.5.1.1. a. in silico factors. Only about a quarter of companies (23%) considered *in silico* approaches to generate information for species selection, with about half (54%) rarely/never considering it. However, a quarter (23%) of the companies not using *in silico* information did state that they should do so in the future. Detailed information about the specific type of *in silico* model/test was not provided.

3.5.1.2. b. in vitro factors

3.5.1.2.1. i. Pharmacology. Of the seven factors listed, tissue cross reactivity was the only one not considered routinely (always/

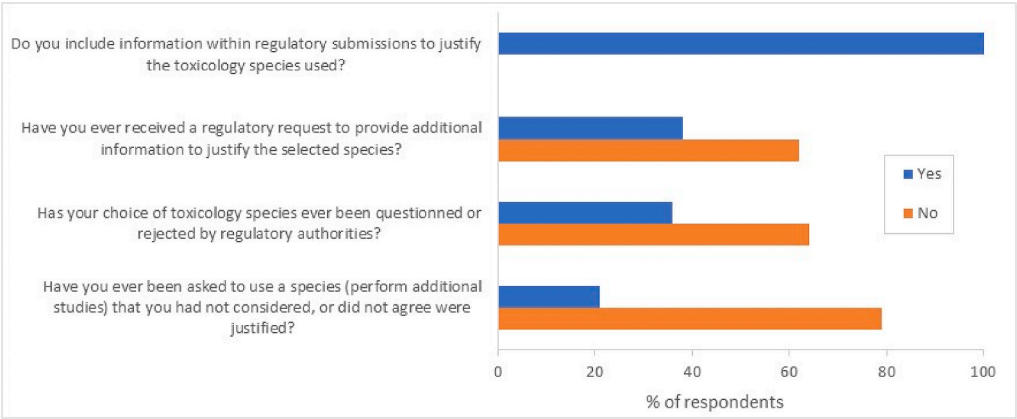

Fig. 1d. Questions regarding Regulatory Interactions. The % of respondents (from 14 total) to separate questions on regulatory interactions/comments regarding species selection. Some respondents provided additional information via free-text comments and examples are included in the results text.

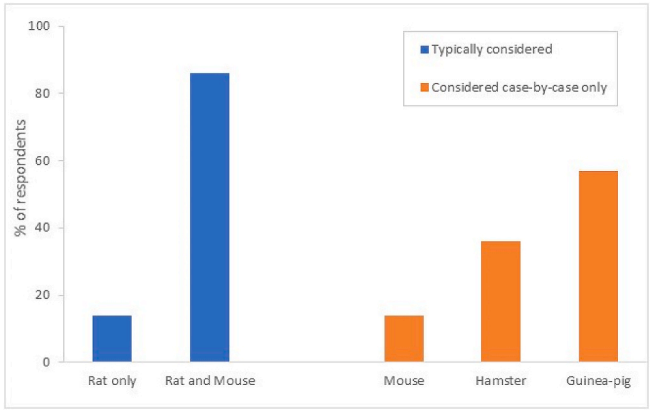

Fig. 2a. Rodent species considered for NCEs. The % of respondents (14 total) that typically consider one or more rodent species for NCE toxicology packages. A separate question asked which rodent species were considered only on a case-by-case basis. Multiple species could be selected.

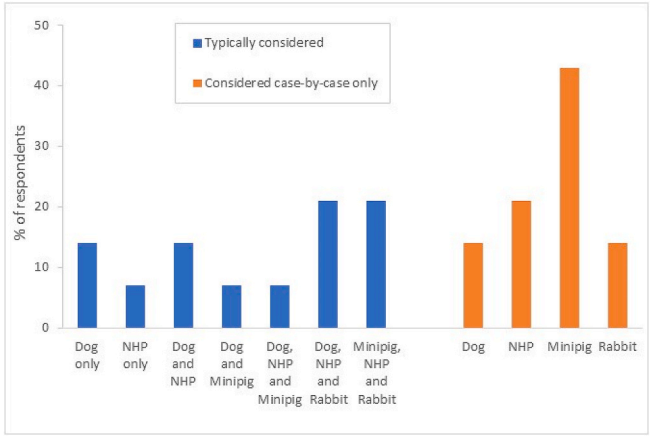

Fig. 2b. Non-rodent species considered for NCEs. The % of respondents (14 total) that typically consider one or more non-rodent species for NCE toxicology packages. A separate question asked which non-rodent species were considered only on a case-by-case basis. Multiple species could be selected. Rabbit is generally considered for embryo-fetal developmental toxicity studies. NHP Non-human primate.

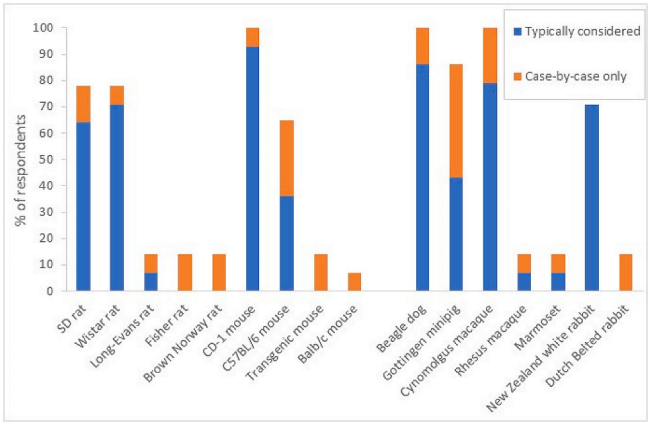

Fig. 2c. Species/Strains/breeds considered for NCEs. The % of respondents (14 total) that typically consider one or more rodent strains or non-rodent species/breeds for NCE toxicology packages. A separate question asked which rodent strains and non-rodent species/breeds were considered only on a case-by-case basis. Multiple strains and species/breeds could be selected. SD is Sprague Dawley rat; Wistar included (Han)Wistar rat.

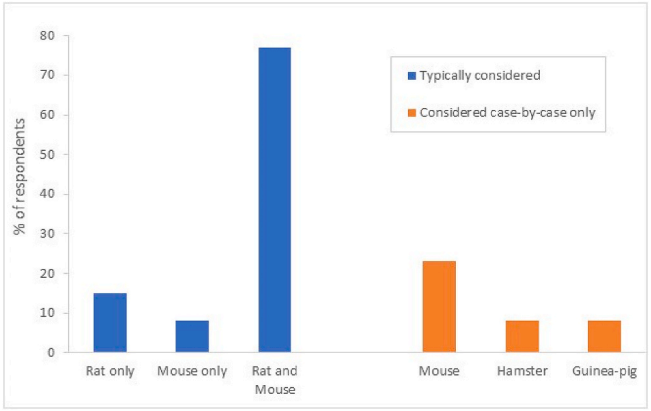

Fig. 3a. Rodent species considered for biologics. The % of respondents (13 total) that typically consider one or more rodent species for biologics toxicology packages. A separate question asked which rodent species were considered only on a case-by-case basis. Multiple species could be selected.

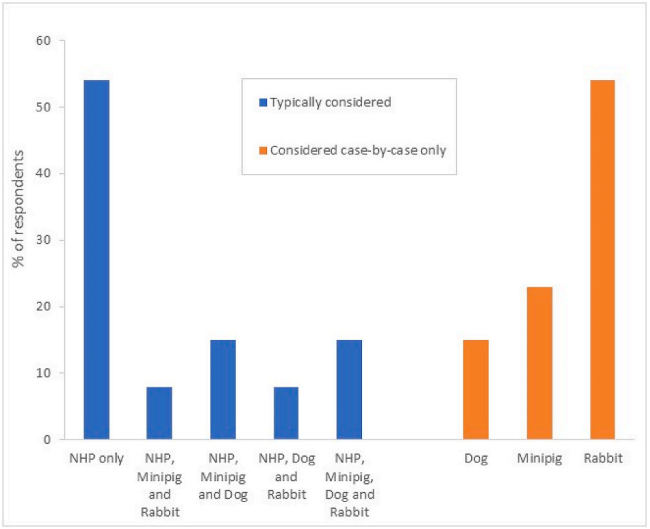

Fig. 3b. Non-rodent species considered for biologics. The % of respondents (13 total) that typically consider one or more non-rodent species for biologics toxicology packages. A separate question asked which non-rodent species were considered only on a case-by-case basis. Multiple species could be selected.

frequently), with the majority of respondents (69%) stating it was rarely/never considered and the remaining stating it was not applicable. Target homology (85%), pathway/mode of action data (85%), target expression (69%), binding affinity/kinetics (62%), functional activity/potency (54%) and tissue distribution of target (54%) were the six factors routinely considered. For these six factors, where not routinely used by the companies/CROs, four of them (pathway/mode of action, target expression, functional activity/potency and tissue distribution of target) were deemed to be useful for possible future consideration by some companies not or rarely using them currently (~8–15%).

3.5.1.2.2. ii. ADME/PK. Of the six factors listed, all were considered routinely (always/frequently), although the least used was tissue/target organ to plasma ratio (15%), with the majority of respondents (77%) stating it was rarely/never considered. The key factors routinely considered were metabolite profile (92%) and similarity of metabolite cross-species (92%), with others including plasma protein binding (77%), clearance (62%) and predicted $T_{1/2}$ (62%). For these six factors where not routinely used, all of them were deemed to be useful for possible future use (~8%).

3.5.1.2.3. iii. Toxicology. Two factors, i.e., cytotoxicity and *in vitro* species-specific toxicity/tolerability (tissue toxicity), were considered. Only about a third of companies (31%) consider cytotoxicity routinely, with the others (69%) rarely/never using it and none of these were considering it for future use. A large proportion of companies (69%) consider species-specific toxicity/tolerability, and of those rarely/never considering (23%), there was interest to consider it in the future (8%).

3.5.1.3. c. in vivo factors

3.5.1.3.1. i. Pharmacology. Of the eight factors listed, whilst overall they were considered routinely, the selected ones varied among companies. Degree of PK/PD data correlation (77%) and demonstrated efficacy in a PD model (62%) were the most considered. The other main factors considered included efficacy translatability of *in vivo* models (46%), existence of biomarkers of disease (38%), availability of disease models (31%), efficacy model for indication expansion (23%), and ease of creating transgenic/knockout models (15%). Of these seven factors, even when not routinely or rarely/never used, all were deemed useful for possible future use (~8–15%), but two of them, ease of creating transgenic/knockout models and efficacy model for indication expansion, were considered not applicable by a small proportion of companies (8–15%). Ease of establishing a minimal anticipated biological effect level (MABEL) was the least routinely used (8%), with the majority not using it (69%), though some organisations reported it may be considered (15%).

3.5.1.3.2. ii. ADME/PK. Of the six factors listed, all were considered routinely, with bioavailability the one key factor used by all companies. Clearance/$T_{1/2}$ (77%), PK profile shape (69%), dose proportionality (54%) and male and female PK difference (46%) were routinely used by

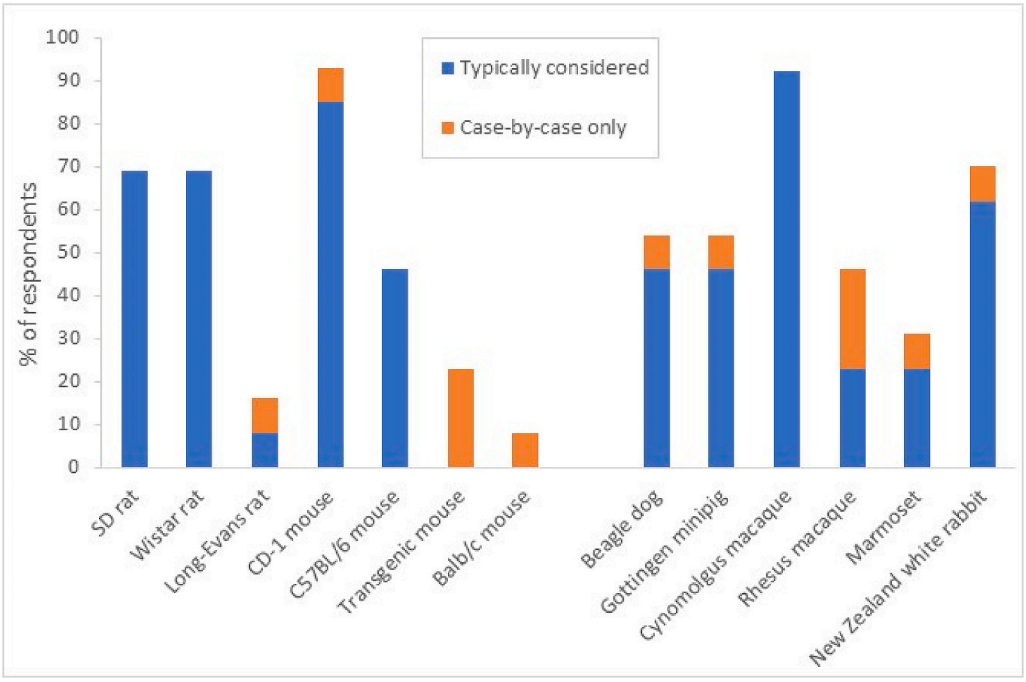

Fig. 3c. Species/Strains/breeds considered for biologics. The % of respondents (13 total) that typically consider one or more rodent strains or non-rodent species/breeds for biologics toxicology packages. A separate question asked which rodent strains and non-rodent species/breeds were considered only on a case-by-case basis. Multiple strains and species/breeds could be selected. SD is Sprague Dawley rat; Wistar included (Han)Wistar rat.

more than half of companies, with some (23%) considering tissue/target organ to plasma concentration/exposure ratio too. Of the three factors not routinely or rarely/never used i.e., clearance/$T_{1/2}$, sex-related PK difference and the tissue/target organ to plasma ratio, some organisations deemed them useful for possible future use (~8–15%).

3.5.1.3.3. iii. Toxicology. This group of factors was the largest of all, with 15 in total. Of these, seven were used routinely by the majority of companies, including route of administration suitability (100%), sensitivity/tolerability and nature of toxicity, historical/background data, anatomical and physiological relevance, formulation/excipient tolerability and ethical considerations (all 92%), and relevance of toxicology findings to human (85%). A further seven factors were used routinely by more than half of companies. These included exposure at Maximum tolerated dose (MTD), Lowest observed adverse effect level (LOAEL)/No observed adverse effect level (NOAEL) (69%), test article requirements (69%), blood volumes (62%), combining safety pharmacology into toxicology studies (62%), available safety pharmacology models (54%), potential for juvenile toxicology (54%) and ease of twice a day (BID) administration (46%). Finally, the need for an alternative route of administration was least routinely considered (38%), with about half rarely/never using it (54%).

3.5.2. Biologics

3.5.2.1. a. in silico factors. Only about a third of companies (33%) considered *in silico* information in species selection, with the other companies (67%) rarely/never considering. No specific information was provided regarding to the type of *in silico* models/tests.

3.5.2.2. b. in vitro factors

3.5.2.2.1. i. Pharmacology. Of the seven factors listed, target homology (100%), pathway/mode of action data (92%), target expression (100%), binding affinity/kinetics (100%), functional activity/potency (83%) and tissue distribution or target (67%) were the six main factors routinely considered. Tissue cross reactivity was the least used routinely, with just under half of companies (42%) stating it was used always/frequently, and the others (58%) stating it was rarely/never considered. Where not routinely used i.e., functional activity/potency, it was deemed useful for possible future use by a few respondents (~8%).

3.5.2.2.2. ii. ADME/PK. Of the five factors listed, all were considered routinely (always/frequently), but the level of use was variable. The main factors routinely considered were predicted $T_{1/2}$ (50%), clearance (42%), metabolite cross-species (25%) and plasma protein binding (25%). The least used was tissue/target organ to plasma ratio (8%), with the majority (75%) stating it was rarely/never considered, and others (17%) it was not applicable.

3.5.2.2.3. iii. Toxicology. Two factors, cytotoxicity and species-specific toxicity/tolerability were considered. Only about a quarter of companies (25%) consider ed cytotoxicity routinely, with the others (75%) rarely/never using it and none of these considering it for future use. Slightly less than half of companies (42%) considered species-specific toxicity/tolerability, with the remainder rarely/never considering it (58%), and none stating interest to consider it in the future.

3.5.2.3. c. in vivo factors

3.5.2.3.1. i. Pharmacology. The majority (7) of the pharmacology-related factors were considered routinely, ranging from just under half, to about three quarters of companies. These factors included degree of PK/PD data correlation (75%), translatability of *in vivo* models (75%), demonstrated efficacy in a PD model (67%), availability of disease models (67%), establishing MABEL (50%), ease of creating transgenic/knockout models (42%) and existence of biomarkers of disease (42%). One factor, efficacy model for indication expansion, was the least used or considered not applicable by three quarters of companies (75% and 8% respectively).

Four factors, translatability of *in vivo* models, ease of creating transgenic/knockout models, efficacy model for indication expansion and efficacy in a PD model, where not routinely or rarely/never used, but were considered to have potential for future use (~8–17%).

3.5.2.3.2. ii. ADME/PK. Of the six factors listed, five were considered routinely, including bioavailability (75%), Clearance/$T_{1/2}$ (75%), dose proportionality (67%), PK profile shape (58%), and male and female PK difference (33%). Tissue/target organ to plasma ratio was rarely/never used by the majority (92%).

3.5.2.3.3. iii. Toxicology. Of the fifteen factors, all were used. Of these, eleven were used most often by about three-quarters of companies and included suitability of route of administration (100%), sensitivity/tolerability and nature of toxicity, relevance of toxicology findings to human, anatomical and physiological relevance, historical/background data, formulation/excipient tolerability (all at 92%), exposure at MTD/LOAEL/NOAEL, combining safety pharmacology into toxicology studies and ethical considerations (83%), blood volumes (75%) and test article requirements (73%). The other factors were still used quite regularly, albeit to a lesser extent. These included an alternative route of administration (50%), available safety pharmacology models (33%), and potential for juvenile toxicology (33%). Ease of BID administration was the least considered factor (17%) with the majority (83%) rarely/never using it.

3.6. Industry insights and case-studies

Here we briefly describe the principles and/or case-examples provided by five companies to highlight similarities and differences in approaches towards species selection for toxicology programs.

3.6.1. General approach to species selection

A brief and general example of the species selection approach is provided by Roche. Species selection is an important part of an overall project strategy discussion and is initiated as early as the target selection phase. There is no formal documentation for species selection, but justification is documented in the form of a slide as part of the strategy presentation to governance boards and/or in study protocols for the respective toxicity studies. At Roche, minipig or cynomolgus monkey were the non-rodent species of choice for many years. However, use of any of the non-rodent species, including dog, for toxicity testing requires the same appropriate scientific justification.

Great emphasis is put on application of the 3Rs principle (Replace, Reduce, Refine) and the human relevance of the animal species, i.e. trying to minimize animal use and maximize the likelihood of identifying responses that are similar to those expected in humans. At target assessment, non-rodent species selection is based on evidence from the literature and databases, including in-house databases allowing genome-based characterization of the different animal species for potential use. For small molecules, it is recommended to clone the target from animal species used (if not commercially available) for binding/functional assays and to establish functional assays to compare on-target activity, if feasible. This information is needed for informed selection of the animal species to assure human relevant safety assessment, avoiding production of potentially irrelevant findings, and potency assessment for safety margin calculation for human starting dose. A model based approach is used for human dose prediction. *In vitro* potencies in animals and human are integrated with other pre-clinical data (e.g. free fractions in plasma and *in vivo* pre-clinical efficacy data) to project the target for efficacy in human. Human PK is forecasted using physiologically based PK modelling and safety margins are estimated by comparing exposures at the NOAEL to the predicted human efficacious exposure.

The testing of biotechnology-derived products in non-relevant species may give rise to misinterpretation and is not recommended. Where no relevant species exists, the use of homologous proteins or the use of relevant transgenic or humanised animals expressing the human target

may be considered. In this context, the use of *in vitro* human cell systems or human-derived material can provide relevant information about these translational differences and improve the understanding of the relevance of the animal models. However, with increasing complexity of the modalities and targets, *in vitro* only approaches are increasingly applied at Roche, i.e. using relevant human *in vitro* models rather than surrogate molecules or transgenic animal models. Both, generation and characterization of a homologous molecule or transgenic mice will lead to a considerable increase and front loading of development activities. In addition, there might be differences in mechanism of action/pharmacology of a surrogate molecule in animal species compared to the clinical candidate in human. Generally, using a surrogate molecule is not useful for quantitative risk assessment. Development of a relevant transgenic mouse model may be lengthy or not be feasible. If feasible, extensive model characterization will be required and immunogenicity of a clinical candidate in mice will likely hamper interpretation in repeat dose and especially chronic toxicology studies. Therefore, human-based *in vitro* only approaches are being applied at Roche whenever possible.

3.6.2. Process and documentation

The approach taken by Merck KGaA (see Table 3a; 3b and 4) describes a multidisciplinary approach with a clear decision-making process and responsibilities. This also includes preparation of a document to record the final choice of species, with reasons to support it (Table 3a). Furthermore, there is a clearly defined ranking system regarding choice of rodent and non-rodent species, which also is inclusive of flexibility for wider scope for different species where scientifically justified (Table 4).

3.6.3. Species selection for an NCE (case-study 1)

Consideration of *in vitro* metabolism as a key factor for species selection is outlined in this next example from Ipsen. An NCE was selected as a candidate for an oncology indication. *In vitro* and preliminary *in vivo* studies in rats and mice showed promising safety and efficacy profiles. An inter-species (mouse, rat, rabbit, dog, minipig, NHP and human) metabolic profiling of the NCE was performed, in order to select the most relevant animal species for toxicology studies. Although the mouse was the most similar rodent to human based on metabolic profile (Table 5), the rat was selected as the rodent species for general toxicology studies, based on other considerations of: better exposure to parent compound X compared to mice, and availability of more historical background data, in addition to a reasonably similar metabolite profile. As the minipig had the most similar non-rodent metabolic profile compared to human (Table 5), it was chosen as the non-rodent species.

3.6.4. Species selection for an NCE (case-study 2)

A further example from Ipsen reflects the occasional experience of companies being challenged or requested to change their selected species. An NCE intended for the treatment of a neurologic disease was developed in rats and NHPs, with the justification of the use of these species being mainly based on relevant metabolic profiles and availability of disease models, for a previous different disease indication. During a Health Authority (HA) meeting (specific region is not disclosed), questions relating to selection of the animal species were raised, with the HA being reluctant to have further NHP studies supporting development for the new indication and suggesting an alternative species would be more applicable since this HA is not in favour of using NHP for non-clinical safety testing and promotes use of other non-rodent species. However, changing the species at that point of clinical development (Phase 2) was considered to be too late, especially as it raised ethical considerations for conducting additional studies in a different species. The HA subsequently accepted NHPs as the non-rodent species for development of the new indication.

3.6.5. Species selection for an NCE (case-study 3)

A further example for NCE non-rodent species selection was published as a poster at the Society of Toxicology (SOT) annual meeting in 2015 (Papoutsi et al., 2015). Following comparison of data from multiple parameters between dog, NHP and minipig for an orally administered NCE, the dog was the species ultimately selected for a small molecule program.

i. Default species: Dog is initially first choice due to high predictive value for human responses (Olson et al., 2000), well understood physiological and biochemical responses. However, the decision is case-by case based on a number of other factors.
ii. Consideration of protein homology of the target of interest (compared to human): Dog and minipig had comparable homology to rat and mouse; NHP had closest protein homology to human.
iii. Choice for target expression: Data was only available for NHP. Since the majority of other factors indicated the dog was suitable, it was not considered necessary to generate this data for dog or minipig.
iv. Choice for PK and TK: There were no clear differences between dog and NHP in terms of accumulation and elimination. Minipig was not quite similar enough to the other two species.
v. Species used in efficacy model: Efficacy models are not always the best choice for nonclinical toxicology studies, although it can be a consideration in some circumstances.
vi. Tolerability of unusual vehicles: Some information was available for dogs and NHPs and a tolerability study confirmed dog to be suitable using an unusual vehicle for the test compound.
vii. Species used for similar classes of compounds: As compounds from a similar class had been tested using dog in non-clinical

Table 3a
Example from Merck KGaA – company approach to species selection - NCE data requirements and responsibilities for the selection of species for preclinical safety studies.

	Bioinformatics	Pharmacology	Drug Metabolism and Pharmacokinetics (DMPK)	Project Leader	Chemical and Preclinical Safety (Toxicology)
Deliver data on target expression in animal species	**Responsible**	Informed	Informed	Informed	Informed
Deliver data on cross species affinity/activity for the target	Informed	**Responsible**	Informed	Informed	Consulted
Provide Pharmacokinetic data to support design of toxicity study and deliver data (incl. Interpretation) on comparative Metabolic Identification (MetID).	Informed	Informed	**Responsible**	Informed	Consulted
Evaluation of competitive data (including animal species)	Informed	Informed	Informed	**Responsible**	Informed
Selection of the species to be used for preclinical safety studies	Consulted	Consulted	Consulted	Consulted	**Responsible**

[1]The Preclinical Safety member of the project team will drive the selection process.
[2]When relevant (e.g. similar target), data from previous programs or competitors should be included in the evaluation.
[3]Data based proposals are made by the project teams following these guidelines and in alignment with the respective line functions.
[4]Proposals are submitted to the management of Preclinical Safety for approval and to the Merck Discovery Review Committee by the Decision Point Team Leader for a final confirmation.
[5]Preclinical Safety team member, together with Decision Point Team Leader, finalizes the animal species selection document.

Table 3b
Example from Merck KGaA – company approach to species selection - New Biologic Entity (NBE) data requirements and responsibilities for the selection of species for preclinical safety studies.

	Bioinformatics	Drug Disposition and Design–Computational Chemistry and Biology	NBE-Group	Pharmacology	Drug Disposition and Design - Drug Metabolism and Pharmacokinetics NBE	Project Leader	Chemical and Preclinical Safety (Toxicology)
Target expression in preclinical safety species	**Responsible**	Informed	Informed	Consulted	Informed	Informed	Informed
Target affinity in preclinical safety species - modelling	Informed	**Responsible**	informed	Informed	Informed	Informed	Consulted
Target affinity in preclinical safety species – *in vitro* for prioritized species	Informed	Informed	**Responsible**	consulted	Informed	Informed	Consulted
Proof of pharmacological function in preclinical safety species	Informed	Informed	Informed	**Responsible**	Informed	Informed	consulted
Pharmacokinetic and immunogenicity assessment *in vivo*	Informed	Informed	Informed	Informed	**Responsible**	Informed	Consulted
Evaluation of competitive data (incl. Pre-clinical safety species)	Informed	informed	Informed	**Responsible**	Informed	Informed	**Responsible**
Recommendation of the species to use for preclinical safety studies	Consulted	Consulted	Consulted	Consulted	Consulted	Consulted	**Responsible**

Table 4
Example from Merck KGaA – company approach to species selection - ranking of species for preclinical safety studies investigating NCE's.

	Species[a]	Priority NCE	Comments NCE
Rodent	Rat	1	First choice
	Mouse	2	In case rat not suitable
Non-rodent	Dog	1	First choice
	Minipig	2	In case dog not suitable
	NHP	3	In case no other non-rodent species is considered suitable (based on e.g., bioavailability, PK, metabolism and pharmacology considerations)

[a] Other species may be used if justified or for specific studies, i.e. rabbit for reproduction toxicity studies, rabbit for local tolerability of parenteral drugs or guinea pigs for early safety pharmacology studies.

toxicology studies, this was another factor considered in the non-rodent species selection.

3.6.6. Challenges with species selection for other molecules/medical devices

Similar approaches for species selection exist for other molecules, including some which many may think of as medical devices. Here, one company (Bracco Group) provides an example of how species selection for imaging diagnostics molecules tends to generally follow a similar path to NCEs.

Gas-filled microbubbles (MB, typically 1–8 μm in diameter) are widely used in clinical practice as contrast agents in medical imaging. In practice, MBs are mixed with e.g. saline, injected intravenously and imaged by ultrasound systems. From the regulatory perspective, HAs (such as US FDA) consider these agents as drugs or drug products (FDA, 2009) and development often follows ICH M3 (R2) (ICH M3(R2), 2009). Typical interspecies metabolic profiling does not allow appropriate selection of the most relevant animal species for nonclinical safety studies, with non-rodent species selection mainly based on PK (i.e., tissue distribution) and the feedback received from various HAs.

The studies evaluating safety and efficacy of MBs during an imaging session/exam (i.e. in-use product) are often conducted in minipigs due to similarities between humans and pigs in cardiovascular anatomy and coagulation profiles (Schneider, 2002), particularly important as hypotension and bradycardia, even though rare, are well-described adverse effects of these agents (Szebeni et al., 2018) and safety pharmacology studies are required.

3.7. Discussion

The overall objectives of the questionnaire were to 1) capture detailed information on current practices by various pharmaceutical companies and CROs in different jurisdictions, 2) explore similarities and differences between NCEs and biologics with regard to species selection and 3) understand emerging new considerations in species selection. Consequently, a questionnaire was developed to capture relevant information and to our knowledge, this is the first time that a systematic approach has been used to develop a comprehensive list of questions/factors to be considered in toxicology species selection. Furthermore, we propose that Table 2 could be used by companies and perhaps regulatory agencies as a guide for factors to be considered for toxicology species selection. Whilst this list of factors was designed to be comprehensive, there are other considerations which could be added, for current or future use.

Table 5
The metabolism of Compound X in liver microsomes of multiple species.

	M1 (%)	M2 (%)	M3 (%)	M4 (%)	M5 (%)	M6 (%)	M7 (%)	Compound X (%)	% of Compound X metabolised
Mouse	7	33	4	3.5	2	–	9	39	61
Rat (male)	–	7	–	–	–	–	–	83	17
Rat (female)	–	4	–	–	–	–	–	94	6
Rabbit	32	61	7	–	–	–	–	–	100
Dog	2	5	–	–	–	–	–	88	12
Minipig	20	27	2	–	–	–	–	43	57
NHP	42	53	2	–	–	–	–	–	100
Human	12	20	4	–	–	–	5	59	41

M: Metabolite number and % of Compound X metabolised. - not detected.

The responses indicate that species selection involves various functions/disciplines, most often including pharmacology, ADME/PK and toxicology, working together within a specific project team. All respondents indicated that the toxicology function is usually responsible for the final decision on which species will be used and why. This multifunctional approach is important in reflecting the complexity of the factors considered for species selection (Table 2) for regulatory submissions. Some small companies may find it more difficult to adopt a multifunctional approach from within, but often access external resource to provide data or advice to ensure sufficient input to this process in order to minimize or avoid regulatory hurdles upon submission.

Justification of species choice is not only required for regulatory submissions, but also for ethical considerations for the use of human relevant species in nonclinical safety assessment studies. This might imply that there would be clear, consistent, well documented approaches being used and reported. Although some recommendations are outlined within regulatory guidelines (EMEA, 2017; ICH website, 2020) (Tables 1a and 1b), the level of information included within submissions is usually limited (Prior et al., 2020b; Baldrick, 2017), generally restricted to why the species used were deemed relevant, whilst statements around 'de-selection' of other species are not typically included. For example, examination of 35 Investigator Brochures (IBs) for NCEs used to support FIH studies showed that only a small number of IBs included rationale for selection of the rodent and non-rodent species for toxicology testing (Baldrick, 2017). Providing justification for species selection in regulatory documents remains a real challenge.

There are occasions when there is a need to change species during development, and if a wider range of species from which to select from has been systematically explored and captured, the data would be readily available to facilitate these decisions. In the survey responses, most companies stated that they have formal or semi-formal processes to guide or outline the steps or data to facilitate toxicology species selection, which mainly consisted of working practices or templated slide-decks for milestone presentation meetings. It may be useful to implement a standardized formal approach e.g., via SOPs or Best Working Practice, to document and share information/data and to generate a report or statement covering species selection. This would help not only to internally guide and document all the relevant data, but also facilitate regulatory submissions, responses to regulatory inquiries, in-licensing due diligence and partnering discussions.

As expected, all organisations confirmed that they include information within regulatory submissions to justify the toxicology species used, however the survey did not explore the level of detail provided. Just over 60% of the companies surveyed had not received requests to provide additional information to justify the selected species or had the choice of toxicology species questioned or rejected. The fact that enquiries about species selection were not predominant suggests that most of the choices made were acceptable, indicating that the processes used by companies are generally fit for purpose. However, it also indicates a need to be prepared in case of enquiries, so that responses are more efficiently and readily available. Most of the provided examples were related to questions or requests for more information around pharmacological relevance for biologics and/or the use of only one species for biologics chronic toxicology studies (an option available if short-term toxicity studies in two species are similar for molecules following ICH S6 (R1) (ICH S6(R1)). This has occasionally led to requests to perform additional studies in another/different species.

3.8. Species selection for NCEs

The results from the questionnaire confirmed that rat is the rodent species of choice for general and reproductive toxicology studies. The majority of respondents also considered the mouse as a possible rodent species in parallel to rat. It is interesting that despite advantageous test article requirements, use of mouse as one of the major *in vivo* pharmacology model species, and ease of genetic manipulation (e.g., creating disease models), it is not used as first choice more frequently in toxicology studies (Ellenbroek and Youn, 2016). Although the reasons why the rat was most commonly selected were not requested, it is likely related to relative ease of handling, larger blood volume and brain size (Ellenbroek and Youn, 2016). In addition, rats are less easily stressed by human contact, which is of particular significance when repeated handling is required for measuring neurological, cognitive and behavioural endpoints, and are more adaptable for juvenile toxicity studies. Some other rodent species such as hamster, guinea pig, are considered on a case-by-case basis for use when rat (or mouse) are not suitable models. Often time, for embryo-fetal developmental toxicity studies, rabbits are used as a second species. Use of rodents other than rat and mouse, may create some challenges, particularly in terms of conducting reproductive toxicology studies both in terms of animal and background data availability.

Rodent strains most commonly used were (Han)Wistar and SD rat and CD-1 mouse, with a range of other strains considered on a case-by-case basis. The choice of rodent strain was often driven by experience (e.g. available historical data) and/or specific company preferences but may also be determined by animal availability. Interestingly, a couple of respondents indicated they used CD-1 mice for oncology projects and C57BL/6 for other projects but did not provide further explanation. There also appeared to be a regional preference for rat strains, as all the Japanese respondents mainly use SD rats and the majority of Europe/US respondents use (Han)Wistar, though a reason cited was partly due to temporary difficulties sourcing (Han)Wistar rats in Japan. The appropriateness and selection of a specific strain of rodent may also be influenced by the type of toxicology studies and/or previous experience with one strain over another for a specific toxicity finding. For example, most retina findings are more readily detected in pigmented rats (e.g., Long Evans) (Charng et al., 2011) or there may be a preference to use (Han)Wistar rats in general and reproductive toxicity studies in anticipation of using them in carcinogenicity studies due to their better survival rate (Hayakawa et al., 2013). When mice are the appropriate rodent species, the CD-1 mouse is commonly used strain as they have a large body of toxicity data (Annas et al., 2013) and have been used extensively in carcinogenicity testing.

Non-rodent species selection for NCEs usually focusses on the dog or NHP and the responses received confirmed this was also the case in our data set. However, the consideration of which species to screen varies among companies, with some only using one non-rodent species (either dog or NHP), whereas others may screen both, whilst approximately half the respondents include three (e.g., the minipig in addition). When considering which species to use, the majority chose either the beagle dog or cynomolgus monkey, and when minipig was used, the Göttingen breed was the most popular choice. In some regions, ethical considerations limit the use of NHP, depending on the drug candidate/type of molecule (NCEs vs biologics) and/or intended clinical indication/severity of disease. For example, in the EU the use of NHP is restricted to support new drugs for debilitating or life-threatening diseases and only when the purpose of the toxicity study cannot be achieved by the use of species other than NHP (see (2010/63/EU, 2010)). Individual companies (Schaefer et al., 2016) may also have a preference towards use of minipig rather than dog (also see 3.6.1). Justification of non-rodent species selection is a requirement in some countries (e.g., Germany), where it is required specifically to state why dog or minipig could not be used instead of NHP. This implies that screening data for all three species should be available for the decision and documentation. As the survey data shows, this is not always the case for other regions globally, and hence there is some inconsistency in approaches. Overall, there appeared to be a trend toward selecting a more commonly used species (rats and dogs) as "default" species for toxicology testing, as also shown by other recent surveys (Prior et al., 2020b; Baldrick and Reichl, 2021). For example in one survey with 92 NCEs, rat was used for 92% of molecules (with mouse in remaining studies) followed by dog (65%),

NHP (32%) or minipig (1%) (Prior et al., 2020b). In another survey with 41 NCEs, rat was used for 90% of molecules (with mouse in the remaining cases) followed by dog, NHP or minipig (66%, 32% and 2%, respectively) (Baldrick and Reichl, 2021).

3.9. Species selection for biologics

For most companies, rat and mouse are the rodent species of choice, although a small proportion only considered either one of them. The use of other rodent species is rare, and on a case-by-case basis. The rodent strains most commonly used are the (Han)Wistar and/or SD rat and CD-1 mouse, with other strains considered when necessary. For most biologics, the range of screening work to choose rodent species is similar to the screening process employed with NCEs, albeit with some clear differences that will be explored later in the discussion.

Non-rodent species selection responses confirmed that the NHP is the primary choice, either as the only species considered (about half of respondents) or in combination with dog and/or minipig and/or rabbit. Minipig and dog are not typically (or rarely) included in the screen for assessment of biological activity, probably most often due to experience or knowledge that target homology is too distant or not relevant enough to human. The non-rodent species/breeds, consistent with NCEs, were cynomolgus monkey and beagle dog, with a range of other species/breeds considered when required (e.g., rhesus monkeys).

This questionnaire did not include specific questions regarding use of transgenic animals if a suitable conventional species was not appropriate e.g. had no target epitope. Transgenic models are to be considered in certain circumstances, recognising that often there is not one available, or perhaps making such a model may be too challenging. If transgenic models are available, selecting the most suitable model, and using them to assess safety in a standard toxicology package could be limiting and difficult. The major limitations are lack of historical data, translatability, immunogenicity, restricted study designs and potentially cost.

3.10. Tests/factors considered for species selection

It is apparent that there is great similarity between NCE and biologics in the process leading to species selection, albeit there are also some differences based on the guideline requirements and nature of molecules. Overall, the role of ADME e.g., *in vitro* metabolism profile, stands out as being irrelevant for biologics, but otherwise all of the factors are used to similar or varying degrees for both types of molecules.

3.11. In silico considerations

Overall, the use of *in silico* techniques would seem to be fairly low (23% for NCEs and 33% for biologics), when one considers that information technology, data science, data mining and modelling tools have advanced significantly and are further advancing rapidly. This suggests that there is still much to be done to integrate these disciplines into the species selection process and to understand applications of these techniques in other areas of drug development.

3.12. In vitro considerations

3.12.1. Pharmacology

The key factors considered by all or the majority of companies for NCEs and biologics were target homology, pathway/mode of action data and target expression, with a high proportion also using binding affinity/kinetics, functional activity/potency and tissue distribution of target. Target homology is important as it increases the probability of data translation from species with the high degrees of homology. Tissue cross reactivity (TCR) was rarely or never used (or considered not applicable) by the majority for NCEs, though for biologics this was used always/frequently by a just under half of the companies. The latter observation is likely due to the fact that a TCR study is not always technically feasible if the new drug candidate is not a good immunohistochemical reagent. Overall, TCR is more commonly used for biologicals likely for better understanding of target biology (e.g., target distribution and potential unexpected binding) and since the mechanism of action of some biologicals might raise potential concern regarding carcinogenicity.

3.12.2. ADME/PK

ADME, in this case metabolite profiling, is known not to be applicable to biologics, but as expected, metabolite profiling and cross-species profiling were the factors used by most companies for NCEs. In alignment with suggested approaches in ICH guidance (ICH M3 (R2), ICH S3A) (ICH M3(R2), 2009; (ICH S3a), 1994) there was also high use of plasma protein binding, clearance and predicted $T_{1/2}$ for NCEs, and whilst these also applied to biologics, the most commonly used (though not by all companies), were clearance and predicted $T_{1/2}$. Tissue/target organ to plasma ratio was the least used factor for NCEs and biologics. Metabolite profiling is important to ensure adequate coverage of human metabolites by toxicology species and is one of the aspects covered in the regulatory guidelines; the strategy may differ between companies (Timmerman et al., 2016). Despite the fact that 92% of respondents indicate inclusion of metabolite profiling, including cross species comparison (see Table 2), the number of animal species included in the ADME/PK assessment is often limited. As such, the most appropriate animal species, i.e. the one with a metabolite profile closest to human, can be missed. In particular, the minipig is rarely routinely considered (Fig. 2b), which is surprising, as metabolites can be readily investigated in several *in vitro* matrices, including hepatocyte preparations, subcellular fractions of liver (microsomes, S9), recombinant enzymes, most of which are commercially available.

3.12.3. Toxicology

Cytotoxicity and species-specific (tissue) toxicity/tolerability were both considered for NCEs and biologics. About a third of companies consider cytotoxicity routinely, whilst a larger percentage of companies include species-specific (tissue) toxicity/tolerability for NCEs, and occasionally for biologics.

3.13. In vivo considerations

3.13.1. Pharmacology

With regard to pharmacology, of the eight factors included in the questionnaire, PK/PD data correlation and demonstrated efficacy in a PD model were the two factors considered by a high proportion of companies for both NCEs and biologics. These data indicate that not only the efficacy but also demonstrating exposure-response data are equally important for species selection since the evaluation of safety margins are often on the basis of the NOAEL and predicted human level exposures. The PK-PD relationships also facilitates determination of the MABEL. The availability of disease models, translatability of *in vivo* models, ease of creating transgenic/knockout models and existence of biomarkers of disease were considered by a higher proportion of companies for biologics compared with for NCEs. Establishing MABEL was not routinely considered by most companies for NCEs but was a factor for biologics (~50% respondents). This is likely due to the fact that the MABEL is typically lower than the NOAEL and higher safety precautions typically considered for biologics due to potentially greater risk of unwanted immunogenicity with biologics. However, for NCEs it is consistent with previous reports indicating infrequent use of MABEL in general, with preference to using Pharmacologically Active Dose (Baldrick and Reichl, 2021).

3.13.2. ADME/PK

Five of the six factors were commonly used/considered for NCEs and biologics, which included bioavailability, clearance/$T_{1/2}$, PK profile

shape, male and female PK difference, and dose proportionality. Tissue/Target organ to plasma ratio was the factor used to the least degree for NCEs and biologics. The exact reason for this observation is not readily available; however, it could be related to several factors: a) perhaps with the exception of paediatric programs these data may not play a direct role in species selection, b) this type of data may not provide accurate information regarding the drug concentration at the site of action e.g., receptors, c) often requires additional animals since euthanasia is required for tissue harvesting and d) requirement for development and qualification of additional bio-analytical methods for tissues. Nevertheless, in certain areas of drug development (e.g., neuroscience/CNS programs) there could be a greater tendency towards determination of tissue/target organ to plasma ratio (e.g., brain and/or CSF to plasma ratios) since these data may explain species sensitivity or juvenile animal sensitivity compared to adult animals despite similar systemic exposures.

3.13.3. Toxicology

In terms of species selection, this group of factors (15 in total) was the largest of all. Although there were some differences in preferences between NCEs and biologics, a high proportion of companies use most of the factors, including route of administration suitability, sensitivity/tolerability and nature of toxicity, historical/background data, anatomical and physiological relevance, formulation/excipient tolerability, ethical considerations, relevance of toxicology findings to human, MTD/LOAEL/NOAEL, test article requirements, blood volumes, and feasibility of combining safety pharmacology into toxicology studies. Important but less frequently considered were availability of safety pharmacology models, potential for juvenile toxicology assessment and feasibility of BID administration. The factor least used routinely for NCEs was the need for an alternative route of administration but for biologics BID administration was the lowest considered factor since these products typically have long or very long terminal elimination $T_{1/2}$.

In practice, due to the complexities of species selection for any molecule, the exact approach will often vary from molecule to molecule. Sometimes, the species selection can be guided in advance based on known unsuitability, for a variety of reasons, without the need for detailed assessment, or from findings in early development toxicology studies such as limitations of emesis which subsequently impacts exposure for orally administered molecules. There are occasions during development when changes are required post species selection, perhaps due to unexpected findings in one or more of the initially chosen species, or when there is a switch of clinical indications. One industry example (see 3.6.4) demonstrated the complexities of changing the clinical indication, where core toxicology studies to support the initial application had been completed. This example highlighted that despite a sound scientific rationale to continue with the same species, one regulatory agency had an alternative view, suggesting a change of species. Therefore, interpretation of data and the existing guidance documents could be a challenging task due to difference of opinion among scientists involved in drug development. In addition to the challenges of balancing the scientific versus regulatory views, it is also important to consider organizational preferences, historical practices and regulatory precedence. Consequently, thorough documentation and standardize processes can be advantageous in streamlining changes in drug development, as well as supporting the original application.

3.14. Concluding remarks

Species selection for nonclinical safety assessment is an involved and complex process and plays a critical role in drug development since information generated is used to conduct human risk assessment and to support clinical development and drug product labelling. This questionnaire has provided valuable data and insightful information on current industry approaches on various aspects of species selection including process and documentation, species/strains/breeds used, and the 'factors' (a comprehensive list of *in silico, in vitro* and *in vivo* tests/data) considered. It confirmed that there are clear similarities for the considerations in species selection for NCEs and biologics across a broad range *in silico, in vitro* and *in vivo* factors. Nevertheless as expected, there are also some differences in focus, with key factors for NCEs as cross species metabolic profile, oral bioavailability and species sensitivity/tolerability whilst key factors for biologics were various *in vitro* pharmacology factors. Furthermore, the extent of use of the various factors was variable across companies, indicating a trend toward very broad approaches, in conjunction with ethical policies for species use.

Selection of species (and strains/breeds) depends on a robust and science-driven process to enable the best translation of nonclinical safety data to humans. In order to increase the likelihood of this translation we should increase the probability of selecting the most appropriate species. This can be achieved by implementing a broad species screening program at early stages of development i.e., inclusion of a wide range of species/strains/breeds that have adequate background/historical data. This ought to include various strains of rodents and minipig during early screening programs, particularly for target characterization (receptor/subtypes expression, homology), *in vitro* metabolic profile and plasma protein binding. The survey results indicated that there are some company and regional differences in species selection approaches, particularly in selection of rodent strain (e.g. (Han)Wistar vs SD rats) or non-rodent species (e.g., minipig vs dog or NHP), which may be influenced by regional ethical considerations, species/strain/breed availability and/or historical bias. These outcomes are similar to those recently reported (Prior et al., 2020b), where potential differences between company approaches and weighting of factors were described. These publications clearly highlight the lack of harmonized approaches in species selection for nonclinical safety assessment even within the same class of molecules (e.g., NCEs or biologics). Although the majority of companies have defined processes and documentation for species selection, the scope of work (e.g., number of species/strains/breeds considered) and style and extent of documentation and reporting are variable. Therefore, it is important to develop consistent and standardized processes to provide greater levels of scientific justification and consistency and overarching reports or documentation, for which this article could serve as a starting point.

CRediT authorship contribution statement

Rostam Namdari: Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Keith Jones:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Samuel S. Chuang:** Writing – review & editing. **Steven Van Cruchten:** Writing – review & editing. **Zuhal Dincer:** Writing – review & editing. **Noel Downes:** Writing – review & editing. **Lars Friis Mikkelsen:** Conceptualization, Writing – review & editing. **Joanna Harding:** Writing – review & editing. **Sven Jäckel:** Writing – review & editing. **Björn Jacobsen:** Writing – review & editing. **Jacqueline Kinyamu-Akunda:** Writing – review & editing. **Andréanne Lortie:** Writing – review & editing. **Sofiene Mhedhbi:** Conceptualization, Writing – review & editing. **Susanne Mohr:** Writing – review & editing. **Michael W. Schmitt:** Writing – review & editing. **Helen Prior:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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