Non-clinical safety studies: comparison ICH M3 – ICH S6

ARGE Univ.-Prof. Dr. Hendrik Ankersmit

23 January 2023

ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals. Step 5, Dec. 2009.

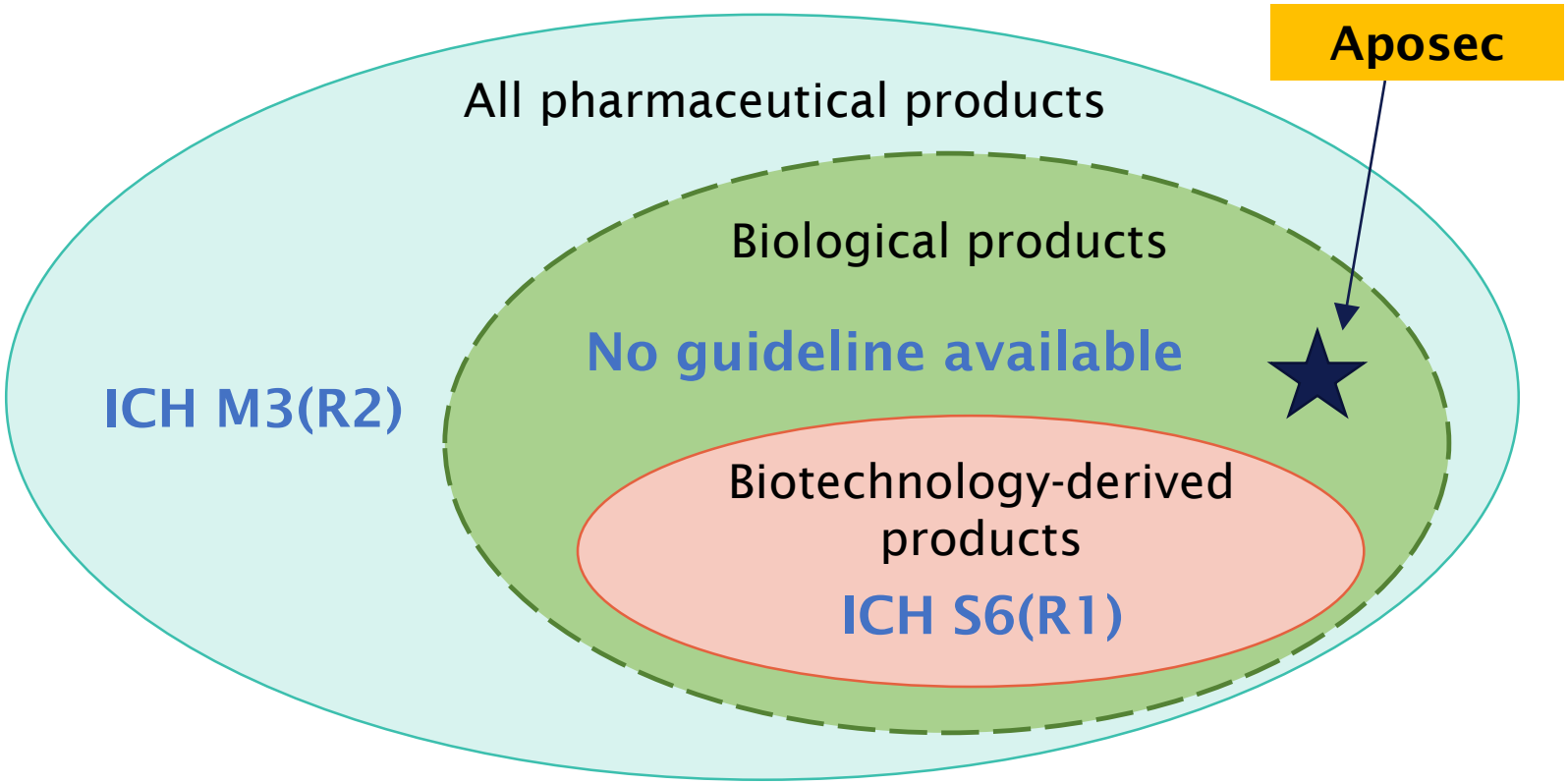
- **Valid for all pharmaceutical products**
- **Valid for clinical trial approval & for market authorisation**

ICH guideline S6(R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals. Step 5, June 2011.

- **Valid just for biotechnology-derived pharmaceuticals**

ICH guideline M3(R2)

ICH guideline S6(R1)



ICH M3(R2)

“Nichtklinische Prüfung (non-clinical testing):

Jegliche Testung eines Prüfpräparats, die nicht am Menschen erfolgt.”

ICH S6(R1)

“Präklinische Prüfung (pre-clinical testing):

Jegliche Testung eines Prüfpräparats, bevor es am Menschen angewandt wird.”

Mag. Robert Klaus: Presentation: „Präklinische Studien für die frühe klinische Prüfungen aus regulatorischer Sicht.“ BASG 2019/03.

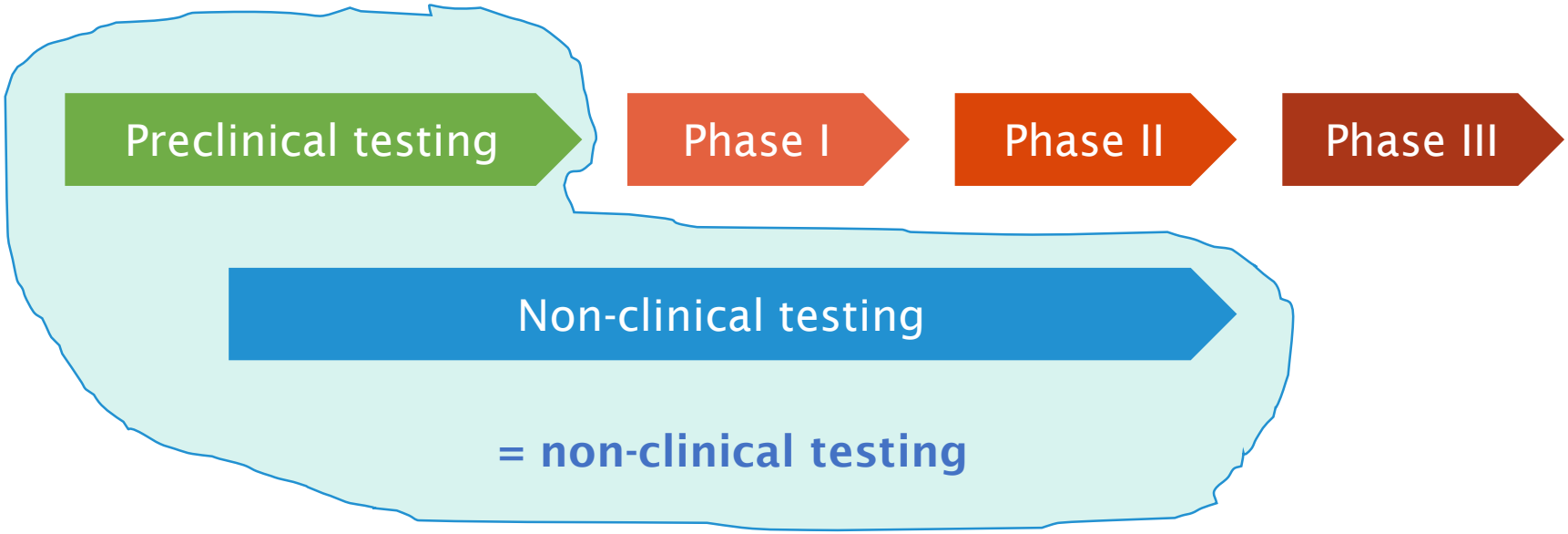
Preclinical testing - a subset of non-clinical testing?

ICH M3(R2)

"Nichtklinische Prüfung" (non-clinical testing):

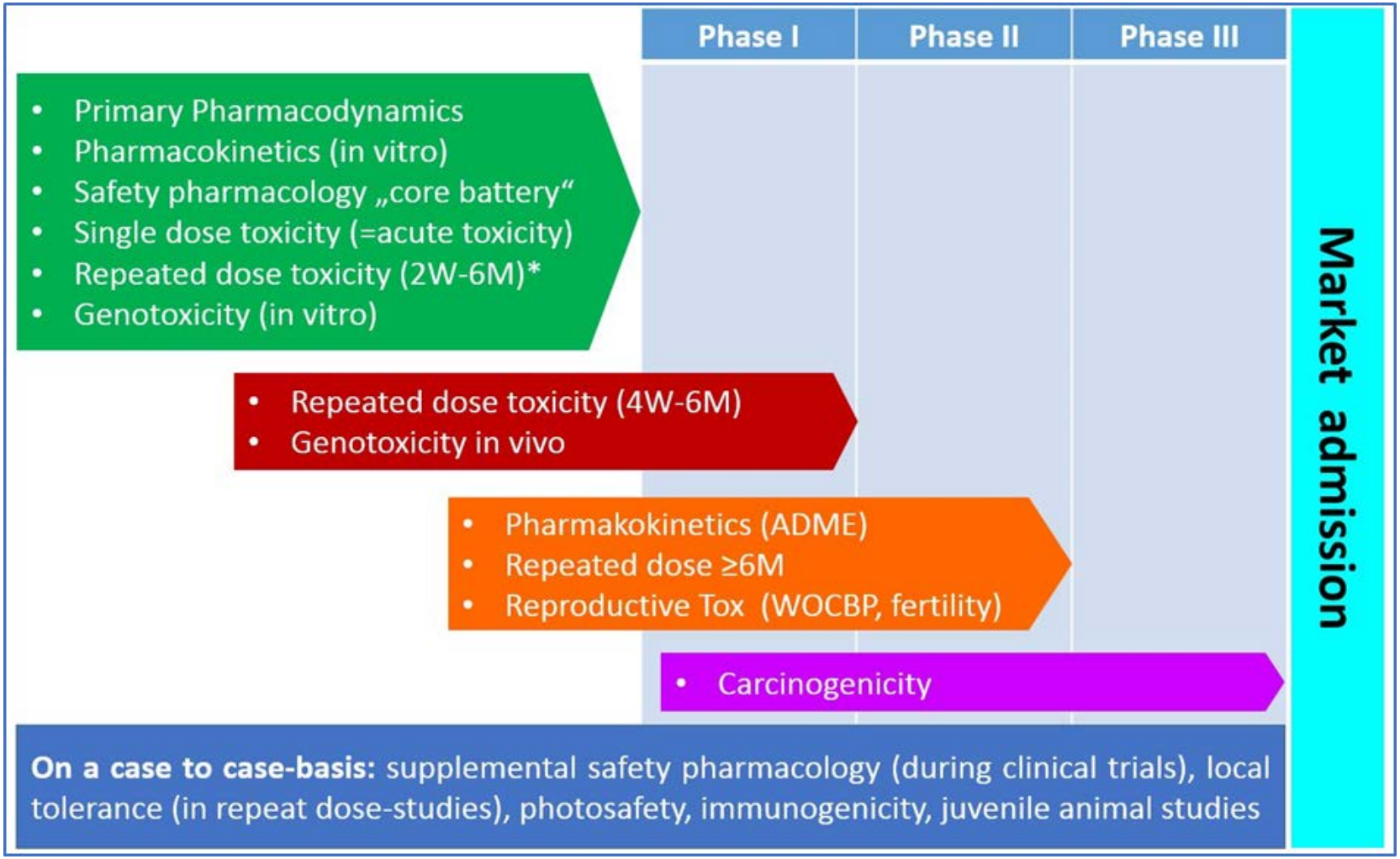
ICH S6(R1)

"Präklinische Prüfung" (pre-clinical testing):

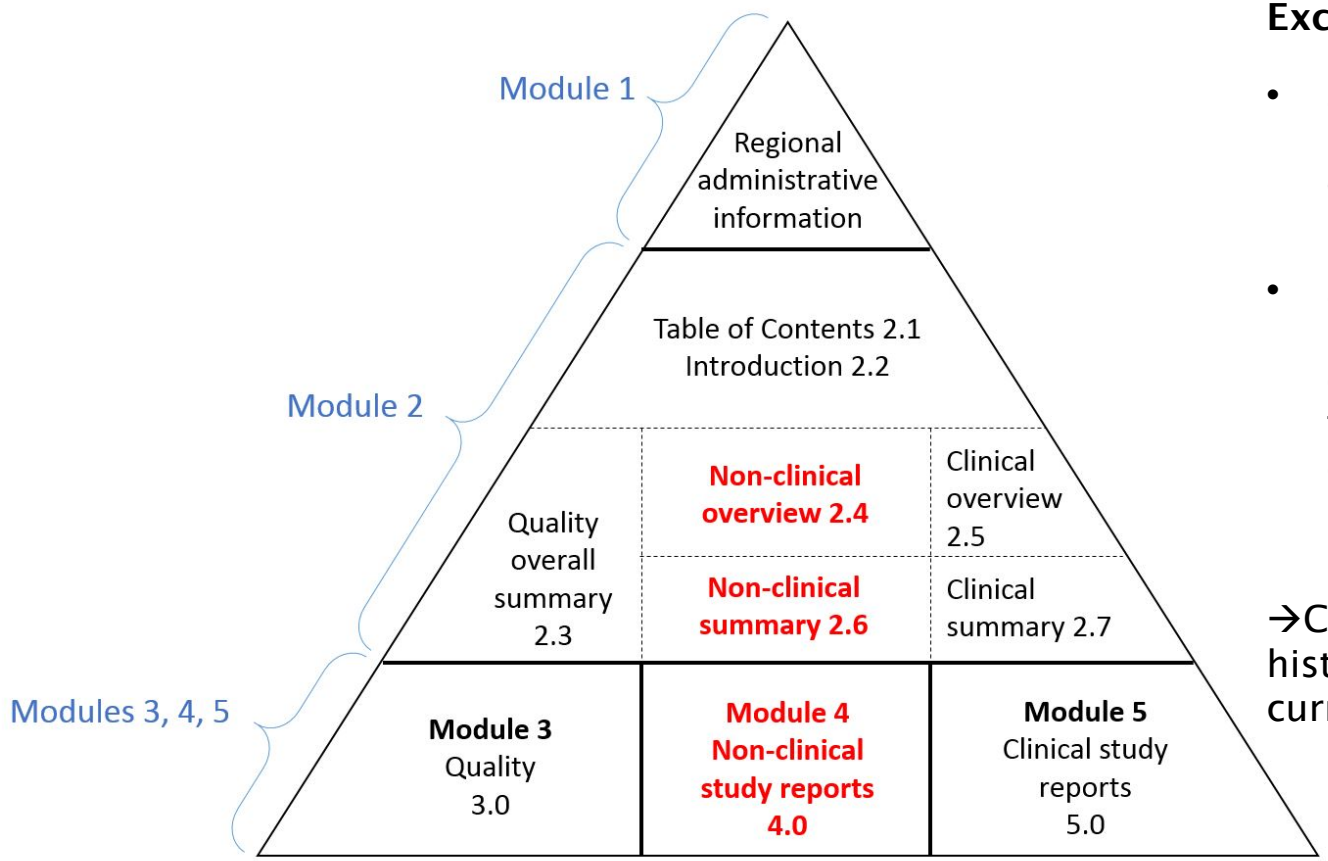


→ Is preclinical testing as subset of nonclinical testing?

Some non-clinical studies are preclinical studies



Preclinical and non-clinical studies in the Common Technical Document



There are no preclinical data in the CTD!
Exceptions:

- Section 1.5.3 – Market Exlusivity: „Significant **pre-clinical** or clinical studies“ are required
- Section 3.2.S.2.6 – Manufacturing Process development: for biotechs the „**developmental process** of the manufacturing process should be provided“ (EMA: Notice for Applicants 2B)

→CTD is not a document on history; it summarizes the current state.

Scope

ICH M3(R2)

"For biotechnology-derived products (as defined in Ref. 1), appropriate nonclinical safety studies should be determined in accordance with ICH S6. For these products, ICH M3(R2) only provides guidance with regard to timing of nonclinical studies relative to clinical development."

Note: Ref.1 = ICH S6(R1)

ICH S6(R1)

"It applies to products derived from **characterised cells** through the use of a variety of expression systems including bacteria, yeast, insect, plant, and mammalian cells. […] The active substances include proteins and peptides, their derivatives, and products of which they are components; they could be derived from cell cultures or produced using recombinant DNA technology including production by transgenic plants and animals.

Examples include but are not limited to: cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones, and monoclonal antibodies."

Scope (2)

ICH M3(R2)

"Pharmaceuticals under development for indications in life-threatening or serious diseases (e.g., advanced cancer, resistant HIV infection, and congenital enzyme deficiency diseases) without current effective therapy also warrant a case-by-case approach to both toxicological evaluation and clinical development…"

ICH S6(R1)

"The principles outlined in this guidance **may also be applicable** to recombinant DNA protein vaccines, chemically synthesised peptides, plasma derived products, endogenous proteins extracted from human tissue, and oligonucleotide drugs.

This document **does not cover** antibiotics, allergenic extracts, heparin, vitamins, cellular blood components, conventional bacterial or viral vaccines, DNA vaccines, or cellular and gene therapies."

Scope: comparison with ICH Q6B

"The principles adopted and explained in this document apply to proteins and polypeptides, their derivatives, **and products of which they are components** (e.g., conjugates). These proteins and polypeptides are **produced from recombinant or nonrecombinant cell-culture expression systems** and **can be highly purified and characterized** using an appropriate set of analytical procedures.

The principles outlined in this document **may also apply** to other product types such as proteins and polypeptides isolated from tissues and body fluids. To determine applicability, manufacturers should consult with the appropriate regulatory authorities.

This document **does not cover** antibiotics, synthetic peptides and polypeptides, heparins, vitamins, cell metabolites, DNA products, allergenic extracts, conventional vaccines, cells, whole blood, and cellular blood components."

ICH Q6B: Specifications: Test procedures and acceptance criteria for biotechnological/biological products. Step 4. 10 March 1999.

Scope: comparison with ICH Q5C

- "The guidance stated in this annex applies to **well-characterised** proteins and polypeptides, their derivatives and **products of which they are components**, and which are isolated from tissues, body fluids, cell cultures, or produced using rDNA technology. Thus, the document covers the generation and submission of stability data for products such as cytokines (interferons, interleukins, colony-stimulating factors, tumour necrosis factors), erythropoietins, plasminogen activators, blood plasma factors, growth hormones and growth factors, insulins, monoclonal antibodies, and vaccines consisting of **well-characterised proteins or polypeptides**. In addition, the guidance outlined in the following sections **may apply** to other types of products, such as conventional vaccines, after consultation with the appropriate regulatory authorities. The document **does not cover** antibiotics, allergenic extracts, heparins, vitamins, whole blood, or cellular blood components."

ICH Q5C: Stability testing of biotechnological/biological Products. Step 4. 30. Nov. 1995

Content

ICH M3(R2) (2009)

- **2. Pharmacology Studies**
- **3. Toxikokinetic and pharmacokinetic studies**
- **4. Acute toxicity studies**
- **5. Repeated dose toxicity studies**
- **6. Estimation of the first dose in human**
- **7. Exploratory clinical trials**
- **8. Local tolerance studies**
- **9. Genotoxicity studies**
- **10. Carcinogenicity studies**
- **11. Reproductive toxicity studies**
- **12. Clinical trials in paedriatic populations**
- **13. Immunogenicity**
- **14. Photosafety testing**
- **15. Nonclinical abuse liability**
- **16. Other toxicity studies**
- **17. Combination drug toxicity testing**

ICH S6(R1) (1997)

3. Preclinical safety testing

3.1. General principles
3.2. Biological activity/pharmacodynamics
3.3. Animal species/model selection
3.4. Number/gender of animals
3.5. Administration/dose selection
3.6. Immunogenicity

4. Specific considerations

4.1. Safety pharmacology
4.2. Exposure assessment
4.2.1 Pharmakokinetics and toxikokinetics
4.2.2 Assays
4.2.3. Metabolism
4.3. Single dose toxicity studies
4.4. Repeated dose toxicity studies
4.5. Immunotoxicity studies
4.6. Reproductive performance and developmental toxicit studies
4.7. Genotoxicity studies
4.8. Carcinogenicity studies
4.9. Local tolerance studies

Content

ICH M3(R2)

ICH S6(R1) ADDENDUM 2008

2. Species selection

2.2. One or two species

2.3. Use of homologous proteins

3. Study design

3.1. Dose selection and application of PK/PD principles

3.2. Duration of studies

3.3. Recovery

3.4. Exploratory clinical trials

4. Immunogenicity

5. Reproductive and developmental toxicity

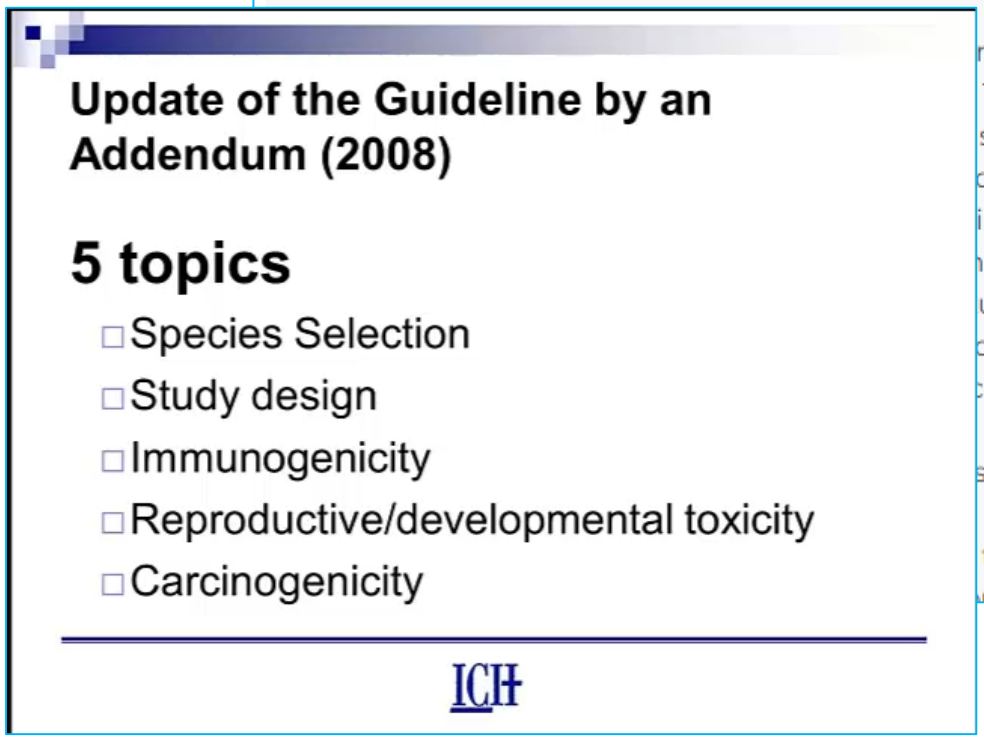
5.2. Fertility

5.3. Embryo-fetal (EFD) and pre/post-natal development (PPND)

5.4. Timing of studies

6. Carcinogenicity

Content: Training Material for S6(R1)



Presentation by Dr. Jan Willem van der Laan, S6 Rapporteur (EU): talks just about the Addendum (2008).

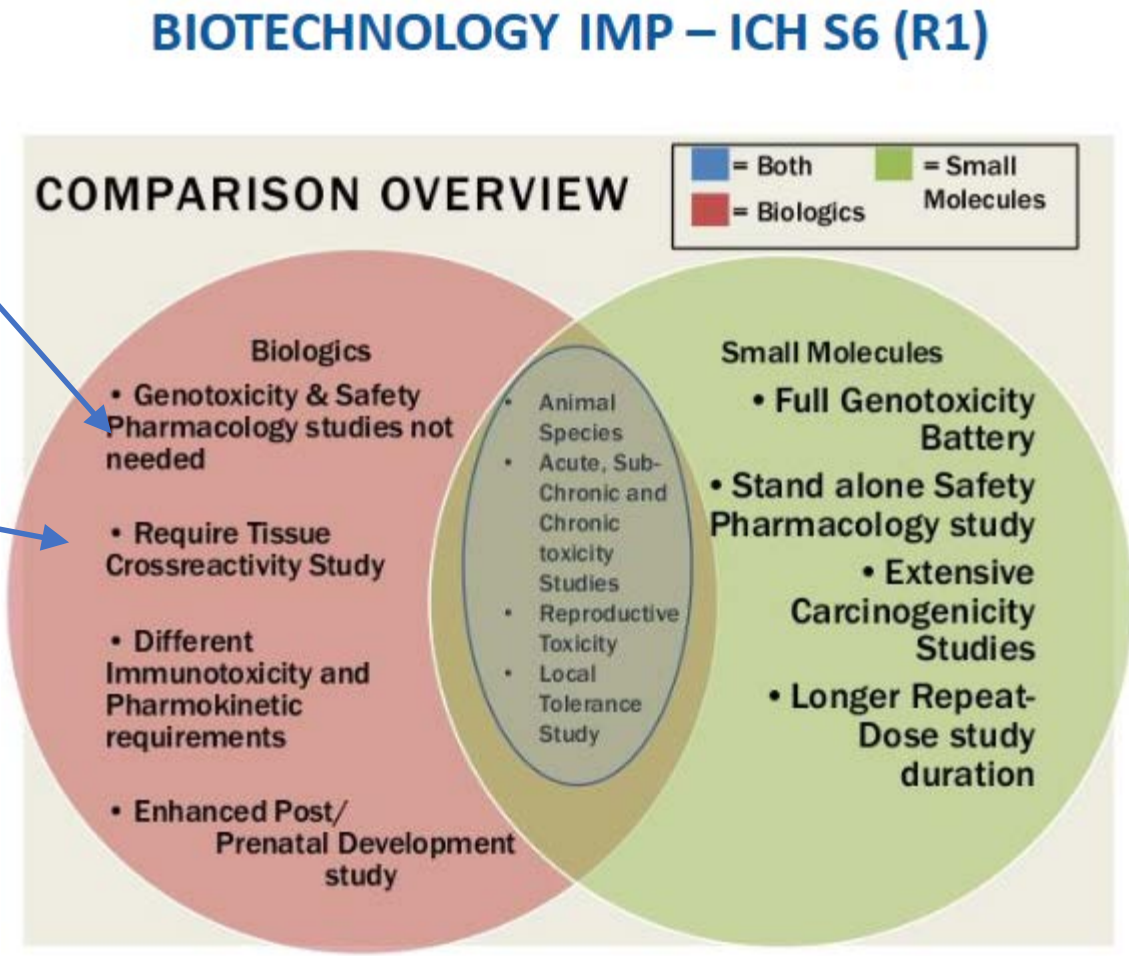
Summary

Topic	Requirement for nonclinical studies for biotechnology-derived products
1. Selection of animal species	Just „relevant“ animal species
2. No. of animal species required	Just one if just one is „relevant“; transgenic animals or homologous proteins if none is relevant
3. Dose selection	High dose only up to 10 times clinical use instead of 50 times
4. Immunogenicity studies	No topic for small molecules (ICH M3), focus on anti-drug-antibodies for biopharmaceuticals
5. Pharmakokinetics: Metabolism	No issue: biotechnology-derived products are catabolised.
6. Duration of repeated dose toxicity studies	Shorter for biotech products
7. Genotoxicity studies	Not needed. Substances do not interact directly with DNA
8. Reproductive and developmental studies	Dependent on patient population; study design needs to be adapted for biotechnology-derived products
9. Carcinogenicity studies	Standard carcinogenicity assays inappropiate; weight of evidence approach if cause for concern

Summary

Safety Pharmacology not needed?

- Should be described for monoclonal antibodies
- Relevant animal species demonstrate similar tissue cross-reactivity



Eva Kolouchova (SUKL): Non-Clinical Research: Key Milestone in Drug Development (STARS Strengthening Regulatory Science Presentation, 23.02.2021)

1. Selection of animal species / model selection

ICH M3(R2)

"In general, the No Observed Adverse Effect Level (NOAEL) determined in nonclinical safety studies performed in the **most appropriate animal species** gives the most important information."

ICH S6(R1)

"Due to the species specificity of many biotechnology-derived pharmaceuticals, it is important to select **relevant animal species** for toxicity testing." (1997)

"**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." (1997)

"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." (1997)

2. No. of animal species required

ICH M3(R2)

"In principle, the duration of the animal toxicity studies conducted **in two mammalian species (one non-rodent)** should be equal to or exceed the duration of the human clinical trials up to the maximum recommended duration of the repeated dose toxicity studies (Table1)."

ICH S6(R1)

"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)." (1997)

"In addition even where two species may be necessary to characterize toxicity in short term studies, it may be possible to justify the use of only one species for subsequent long term toxicity studies (e.g., if the **toxicity profile** in the two species is comparable in the short term)." (1997)

Toxicological profile (definition)

Profile: to take (e.g. 4-8) matrix samples during a dosing interval to make an estimate of C_{max} and/or $C_{(time)}$ and area under matrix concentration-time curve (AUC).

ICH S3A Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies. Step 4, 27 Oct. 1994. Note 1

Alternative definition:
„**toxicological profile:** A summary of the toxic effects of a particular substance, including the levels of exposure at which these effects occur."

https://www.efsa.europa.eu/en/glossary/toxicological-profile
(European Food Safety Authority)

2. No. of animal species required

ICH M3(R2)

ICH S6(R1)

"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**. If the toxicological findings from these studies are similar or the findings are understood from the mechanism of action of the product, then longer-term **general toxicity studies** in one species are usually considered sufficient." (2008)

"The use of **one species** for all **general toxicity studies** is justified when the clinical candidate is pharmacologically active in only one species." (2008)

General toxicity studies (definition)

No ICH, EMA or FDA definition available.

"The general toxicity test is the most basic and essential toxicity test to predict the toxicity of new test substances in advance.
This provides basic toxicity information such as Approximate Lethal Dose (ALD), No Observed Adverse Effect Level (NOAEL), and Maximum Tolerated Dose Finding Study (MTD). This test is divided into a **single (acute) dose toxicity test** and a **repeated dose (sub-acute and chronic) toxicity test**, depending on the duration of the administration."

http://www.ktr.or.kr/eng/test-evaluation/healthcare/contentsid/1023/index.do

[Korea Testing and Research Institute, accessed 21 January 2023]

3. Dose selection

ICH M3(R2)

"To support Phase III clinical trials for the United States, dose-limiting toxicity generally should be identified in at least one species when using the **50-fold margin of exposure** as the limit dose."

ICH S6(R1)

"Dosage levels should be selected to provide information on a dose-response relationship, including a toxic dose and a no observed adverse effect level (NOAEL)." (1997)

"Pharmacokinetic-pharmacodynamic (PK-PD) approaches […] can assist in high dose selection by identifying **1) a dose which provides the maximum intended pharmacological effect in the preclinical species**; and **2) a dose which provides an approximately 10-fold exposure multiple over the maximum exposure to be achieved in the clinic**. The higher of these two doses should be chosen for the high dose group in preclinical toxicity studies…" (2008)

4. Immunogenicity studies

ICH M3(R2)

"Immunogenicity" is mentioned just once in ICH M3, in relation to repeated dose studies up to 6 months in Japan and the USA:

"When immunogenicity or intolerance confounds conduct of longer term studies." – then studies of just 6 months can be appropriate."

ICH S6(R1)

"Many biotechnology-derived pharmaceuticals intended for human are immunogenic in animals. Therefore, **measurement of antibodies** associated with administration of these types of products should be performed when conducting repeated dose toxicity studies in order to aid in the interpretation of these studies" (1997)

"**Antibody responses** should be characterised (e.g., titer, number of responding animals, neutralising or non-neutralising), and their appearance should be correlated with any pharmacological and/or toxicological changes." (1997)

4. Immunogenicity studies

ICH M3(R2)

ICH S6(R1)

"Specifically, the effects of **antibody formation** on pharmacokinetic /pharmacodynamic parameters, incidence and/or severity of adverse effects, complement activation, or the emergence of new toxic effects should be considered when interpreting the data" (1997)

"Attention should also be paid to the evaluation of possible pathological changes related to **immune complex formation and deposition**." (1997)

4. Immunogenicity studies

ICH M3(R2)

ICH S6(R1)

"[Immunogenicity] analyses in nonclinical animal studies are not relevant in terms of predicting potential immunogenicity of human or humanized proteins in humans." (2008)

"Measurement of **anti-drug antibodies (ADA)** in nonclinical studies should be evaluated when there is 1) evidence of altered PD activity; 2) unexpected changes in exposure in the absence of a PD marker; or 3) evidence of immune-mediated reactions (immune complex disease, vasculitis, anaphylaxis, etc.)." (2008)

5. Pharmacokinetics: Metabolism

ICH M3(R2)

"In **vitro metabolic and plasma protein binding data** for animals and humans and systemic exposure data (ICH S3A, Ref. 7) in the species used for repeated-dose toxicity studies generally should be evaluated before initiating human clinical trials."

"Nonclinical characterization of a **human metabolite(s)** is only warranted when that metabolite(s) is observed at exposures greater than 10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Such studies should be conducted to support Phase III clinical trials."

ICH S6(R1)

"The expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation into small peptides and individual amino acids. Therefore, **the metabolic pathways are generally well understood**. Classical biotransformation studies as performed for pharmaceuticals are **not needed**."

=Biopharmaceuticals are not metabolized but **catabolized** (metabolism = anabolism (biosynthesis) + catabolism (breakdown of large molecules into smaller units)

6. Duration of repeated dose toxicity studies

ICH M3(R2)

Contains tables:

Table 1 Recommended Duration of Repeated-Dose Toxicity Studies to Support the Conduct of Clinical Trials:

Maximum Duration of Clinical Trial	**Recommended Minimum Duration of Repeated-Dose Toxicity Studies to Support Clinical Trials**	
	Rodents	Non-rodents
Up to 2 weeks	2 weeks[a]	2 weeks[a]
Between 2 weeks and 6 months	Same as clinical trial[b]	Same as clinical trial[b]
> 6 months	6 months[b, c]	9 months [b, c, d]

Table 2: Recommended Duration of Repeated-Dose Toxicity Studies to Support Marketing:

Duration of Indicated Treatment	**Rodent**	**Non-rodent**
Up to 2 weeks	1 month	1 month
>2 weeks to 1 month	3 months	3 months
>1 month to 3 months	6 months	6 months
>3 months	6 months[c]	9 months [c,d]

n.b. See footnotes c and d in Table 1.

ICH S6(R1)

Does not contain tables (cannot be read alone):

"The duration of repeated dose studies should be based on the intended duration of clinical exposure and disease indication. This duration of animal dosing has generally been **1-3 months** for most biotechnology derived pharmaceuticals." (1997)

"For biopharmaceuticals intended for short-term use (e.g., < to 7 days) and for acute life-threatening diseases, repeated dose studies **up to two weeks** duration have been considered adequate to support clinical studies as well as marketing authorisation." (1997)

"For those biopharmaceuticals intended for chronic indications, studies of **6 months** duration have generally been appropriate…" (1997)

6. Duration of repeated dose toxicity studies supporting clinical trials

	ICH M3		ICH S6
Max. duration of clinical trial	**Min. duration of repeated tox studies**		
	Rodents	**Non-rodents**	
Up to 2 weeks; up 7 days for biopharmaceuticals	2 weeks	2 weeks	2 weeks
2 weeks – 6 months	Same as clinical trial	Same as clinical trial	Same as clinical trial
>6 months	6 months	9 months	6 months, should be scientifically justified

6. Duration of repeated dose toxicity studies supporting marketing authorisation

	ICH M3		ICH S6
Max. duration of clinical trial	**Min. duration of repeated tox studies**		
	Rodents	**Non-rodents**	
Up to 2 weeks; up 7 days for biopharmaceuticals	1 month	1 month	2 weeks
>2 weeks – 1 month	3 months	3 months	1 month*
>1 month – 3 months	6 months	6 months	3 months*
>3 months	6 months	9 months	6 months, should be scientifically justified

* "The duration of repeated dose studies should be based on the intended duration of clinical exposure and disease indication. This duration of animal dosing has generally been **1-3 months for most biotechnology-derived pharmaceuticals**." (ICH S6)

7. Genotoxicity studies

ICH M3(R2)

"An assay for **gene mutation** is generally considered sufficient to support all single dose clinical development trials."

"To support multiple dose clinical development trials, an additional assessment capable of detecting **chromosomal damage** in a mammalian system(s) should be completed…"

"A **complete battery of tests for genotoxicity** should be completed before initiation of Phase II trials."

ICH S6(R1)

"The range and type of genotoxicity studies routinely conducted for pharmaceuticals are **not applicable** to biotechnology-derived pharmaceuticals and therefore are **not needed**." (1997)

"Moreover, the administration of large quantities of peptides/proteins may yield uninterpretable results. It is **not expected that these substances would interact directly with DNA** and other chromosomal material (Note 3)."

8. Reproductive and developmental studies

ICH M3(R2)

"All female reproduction toxicity studies (Ref. 3) and the standard battery of genotoxicity tests (Ref. 10) should be completed before inclusion, in any clinical trial, of WOCBP not using highly effective birth control (see Note 3) or whose pregnancy status is unknown."

WOCB – Women of child-bearing potential

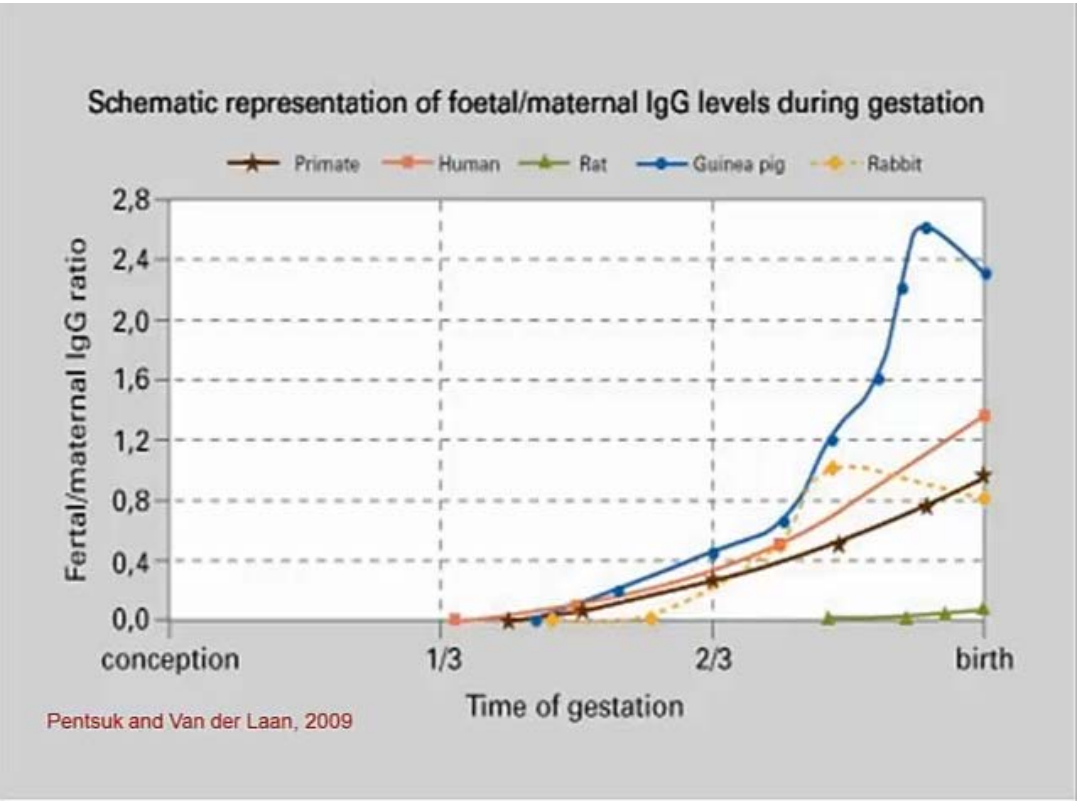
- Fertility studies (can be evaluated in repeated dose toxicity studies)
- Embryo-fetal development
- Pre/post-natal development

ICH S6(R1)

"The need for reproductive/developmental toxicity studies is dependent upon the product, clinical indication and intended patient population (Note 2)." (1997)

"High molecular weight proteins (>5,000 D) do not cross the placenta by simple diffusion. For monoclonal antibodies with molecular weight as high as 150,000 D, there exists a specific transport mechanism, the neonatal Fc receptor (FcRn) which determines fetal exposure and varies across species." (2008, Note 3)

8. Reproductive and developmental studies



Presentation by Dr. Jan Willem van der Laan, S6 Rapporteur (EU):
Placental transfer of monoclonal antibodies:

- IgG1 is most efficiently transported, IgG2 least efficiently
- 1st and 2nd trimester low transfer for all classes.

9. Carcinogenicity studies

ICH M3(R2)

"If carcinogenicity studies are recommended for the clinical indication, they should be conducted to support the marketing application."

ICH S6(R1)

"Standard carcinogenicity bioassays are generally **inappropriate** for biotechnology-derived pharmaceuticals." (1997)

"However, product-specific assessment of carcinogenic potential may still be needed depending upon **duration of clinical dosing, patient population and/or biological activity of the product** (e.g., growth factors, immunosuppressive agents, etc.)" (1997)

9. Carcinogenicity studies

ICH M3(R2)

"Only in circumstances where there is a **significant cause for concern** for carcinogenic risk should the study results be submitted to support clinical trials."

"For pharmaceuticals developed to treat certain serious diseases for adults or paediatric patients, carcinogenicity testing, if recommended, can be concluded post-approval."

ICH S6(R1)

"When there is a **concern about carcinogenic potential** a variety of approaches may be considered to evaluate risk." (1997)

"Products that may have the **potential to support or induce proliferation of transformed cells and clonal expansion possibly leading to neoplasia** should be evaluated with respect to receptor expression in various malignant and normal human cells that are potentially relevant to the patient population under study." (1997)

9. Carcinogenicity studies

ICH M3(R2)

ICH S6(R1)

"The ability of the product to stimulate growth of normal or malignant cells expressing the receptor should be determined." (1997)

"When in vitro data give **cause for concern** about carcinogenic potential, further studies in relevant animal models may be needed." (1997)

9. Carcinogenicity studies

ICH M3(R2)

ICH S6(R1)

"If the weight of evidence (see above) **supports the concern** regarding carcinogenic potential, **rodent bioassays are not warranted**. In this case potential hazard can be best addressed by **product labeling and risk management practices**. However, when the weight of evidence is unclear, the sponsor can propose additional studies that could mitigate the mechanism-based concern…" (2008)

"If the weight of evidence from this more extensive assessment does not suggest carcinogenic potential, **no additional nonclinical testing is recommended**." (2008)

(**Weight of evidence approach:** includes review of pubished data, information on class effects, target biology & mechanism of action, in vitro data, data from chronic toxicity studies & clinical data.)

Regulatory environment: ICH

ICH S1A, 8 pages; S1B(R1), 25 pages; S1C (R2), 12 pages – Carcinogenicity Studies
ICH S2 (R1) – Genotoxicity Studies. 29 pages
ICH S3A – Toxicokinetics. 15 pages. ICH S3B – Pharmacokinetics. 4 pages
ICH S4 – Duration of Chronic Toxicity Testing in Animals. 4 pages
ICH S5 (R3) – Reproductive Toxicology. **120 pages!**
ICH S6(R1) – Biotechnology-derived Products. 23 pages
ICH S7A – Safety Pharmacology. 13 pages
ICH S7B QT – Delayed ventricular repolarization. 14 pages
ICH S8 – Immunotoxicology Studies. 15 pages
ICH S9 – Anticancer pharmaceuticals. 13 pages
ICH S10 – Photosafety Evaluation. 19 pages
ICH S11- Non-clinical safety testing for Paediatric Medicines. 43 pages
ICH S12 (Draft Guideline) – Biodistribution consideration for Gene Therapy Products. 11 pages

Source: https://www.ich.org/page/safety-guidelines

EMA: Non-clinical studies: entry page

https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-guidelines/non-clinical-guidelines

Regulatory environment: EMA

- Guideline on repeated dose toxicity (CPMP/SWP/1042/999 Rev1 Corr*. 18 March 2010)
- Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products (EMEA/CHMP/SWP/28367/07 Rev. 1. 20 July 2017)
- Questions and answers on the withdrawal of the 'Note for guidance on single dose toxicity' (EMA/CHMP/SWP/81714/2010) → Guideline for single dose toxicity was removed; acute toxicity should be integrated into repeat dose toxicity studies

Regulatory environment: FDA

- Guidance for Industry. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (2005)

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m², Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95