The New ICH-Guideline S11 on Nonclinical Safety Testing - Opportunities and Challenges for Overall Development of Paediatric Medicine

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IV. LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ADME	Absorption, Distribution, Metabolism, and Excretion
AE	Adverse Event
CDER	Center for Drug Evaluation and Research
CNS	Central Nervous System
CPK	Kreatincinase
DART	Developmental And Reproductive Toxicity
DB-ALM	Database on Alternative Methods to Animal Experimentation
DLT	Dose Limiting Toxicity
DRF	Dose Range Finding
EDQM	European Directorate for the Quality of Medicines
EMA	European Medicines Agency
EPAR	European Public Assessment Report
EU	European Union
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FDA	Food and Drug Administration
FIH	First-In-Human
GGT	Gamma-Glutamyltransferase
HTA	Health Technology Assessment
ICAPPP	International Council on Animal Protection in Pharmaceutical Programs
ICH	International Council for Harmonistaion of Technical Requirements for Pharmaceuticals for Human Use
IND	Investigational New Drug
JAS	Juvenile Animal Study

JRC	Joint Research Centre
MAH	Marketing Authorisation Holder
MPH	Methylphenidate
mPPND	modified Pre- and Postnatal Development
MPS	Microphysiological Systems
mRDT	modified Repeated-Dose Toxicity
NcWg	Non-clinical Working Group
NHP	Non-Human Primate
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PBPK	Physiologically Based Pharmacokinetic
PDCO	Paediatric Committee
PedRA	Paediatric Record Application
PIP	Paediatric Investigation Plan
PK	Pharmacokinetic
PND	Postnatal Day
PPND	Pre- And Postnatal Development
QALYs	Quality Adjusted Life Years
QSAR	Quantitative Structure–Activity Relationship
R&D	Research & Development
RDT	Repeated-Dose Toxicity
SmPC	Summary of Product Characteristics
US	United States
WHO	World Health Organisation
WoE	Weight of Evidence

Sie wussten nicht,
dass es unmöglich war,
darum machten sie es einfach.

Mark Twain

Demokratie ist nicht einfach da,
sondern wir müssen immer wieder
für sie miteinander arbeiten.

Dr. Angela Merkel
(Auszug aus einer auch insgesamt
bemerkenswerten Rede, gehalten
am 03.10.2021)

1 INTRODUCTION

1.1 Background

With the introduction of paediatric specific legislation, the paediatric drug development has become a compulsory part of the adult drug marketing application in the USA and Europe (unless a preceding waiver has been granted) [1–4]. Since then, a lot of paediatric drug development programmes have been conducted and the availability of medicines specifically developed for children has increased [5]. Simultaneously, the alarming off-label use of pharmaceuticals only approved for adults has decreased.

However, analogous to every success story with each provision of a safe, efficient, and approved paediatric pharmaceutical comes a variety of failed clinical trials and even more numerous nonclinical drug development programmes [6].

Additional nonclinical safety investigations have become more important also due to the enhanced interest in paediatric-only/first indications. The paediatric-first/only development should be supported by additional nonclinical safety investigations to address safety concerns that can usually be clarified by previously conducted clinical trials with adults [7].

There is the consensus within the scientific community, that children are not small adults [8]. Especially new-borns and infants go through an intensive period of growth and development and therefore should be considered completely distinct from the adult population.

For this reason, the overarching objective of the nonclinical part of paediatric drug development process "is to obtain information on the potentially different safety profiles from those seen in adults" [9]. Such differences could be qualitative and/or quantitative, immediate and/or delayed. They could also be caused by

pharmacokinetic/dynamic differences, developmental differences in growth, maturation, and function of target organs/systems [10].

Juvenile animal studies (JAS) are often required as part of the nonclinical safety assessment because they "can be used to investigate findings that cannot be adequately, ethically, and safely assessed in paediatric clinical trials" [9].

With regard to JAS, the *Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*, also called ICH M3, states: JAS "should be considered only when previous animal data and human safety data, including effects from other drugs of the pharmacological class, are judged to be insufficient to support paediatric studies" [11]. However, the ICH M3 does not provide any criteria for this complex decision-making process. Furthermore, conflicting recommendations of further guidelines facilitated the conduct of similar animal studies between regulatory regions without substantial added value and hampered a preferably quick and wide availability of medicines for children [9; 12; 13].

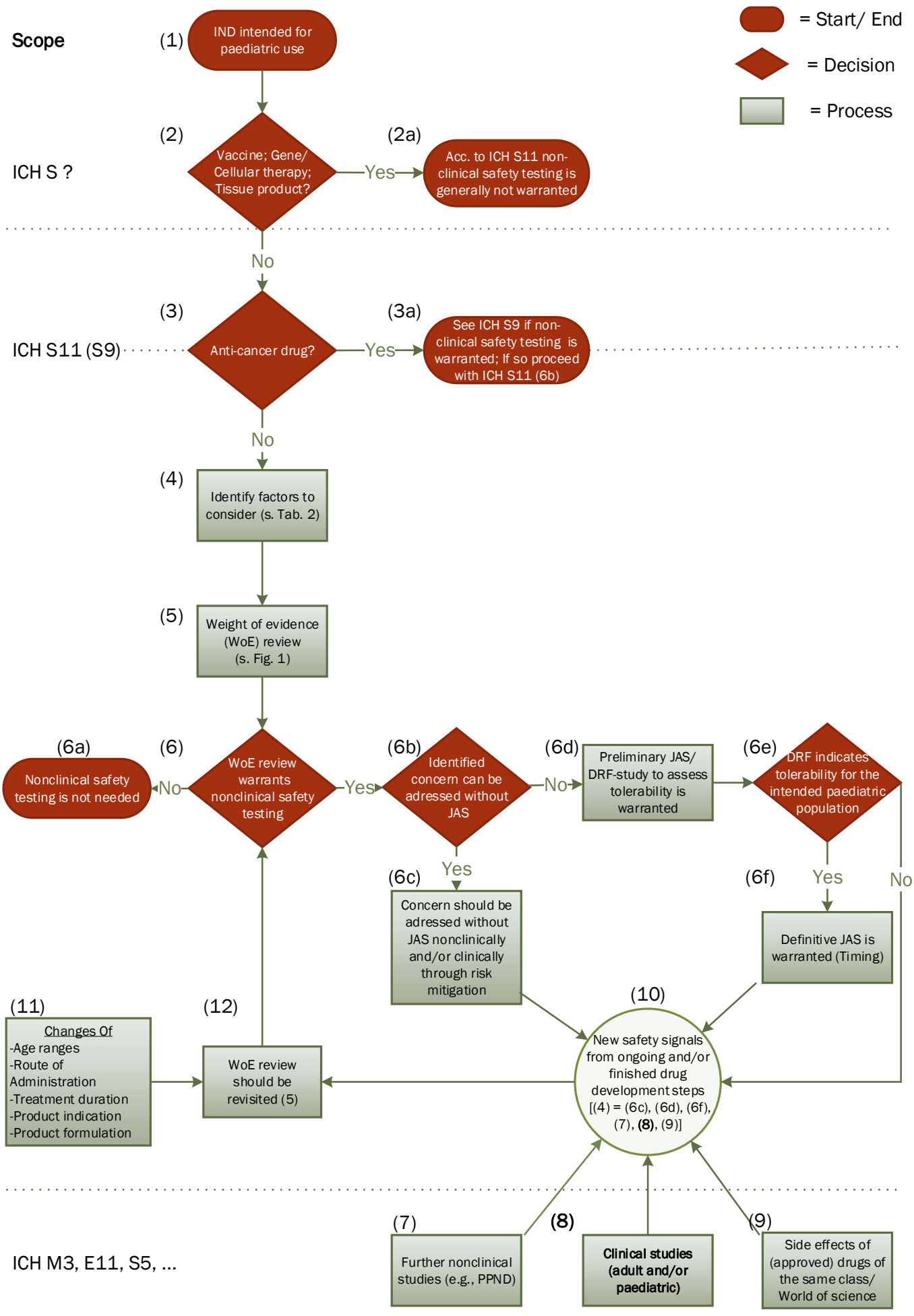
To overcome these challenges (i.e., a lack of further guidance and hamonisation) the ICH issued the new safety guideline *Nonclinical Safety Testing in Support of Development of Paediatric Pharamceuticals,* also called ICH S11 [7]. The self-declared goal of the ICH S11 is the recommendation of "international standards for, and promote harmonisation of, the nonclinical safety assessments to support the development of pharmaceuticals intended for paediatric use."

Essentially the ICH S11 is subject of this work. The following chapter provides an overview of the ICH S11-driven nonclinical drug development. Futhermore, it is intended to underline the importance of the nonclinical part for the overall paediatric drug devlopment process and should serve as a roadmap for the subsequent sections.

1.2 The ICH S11 Driven Decision Process - an Overview

(see Flowchart)

For a general understanding of the ICH-driven paediatric drug development, recommendations of actions issued in different sections of ICH S11' guideline have been presented in the flowchart below. The detailed recommendations to single and yet coherent decisions and operation processes are discussed in subsequent sections.


= Start/ End
= Decision
= Process
Scope
(1)
IND intended for paediatric use
(2)
Vaccine; Gene/ Cellular therapy; Tissue product?
Yes
(2a)
Acc. to ICH S11 non-clinical safety testing is generally not warranted
ICH S ?
No
(3)
Anti-cancer drug?
Yes
(3a)
See ICH S9 if non-clinical safety testing is warranted; If so proceed with ICH S11 (6b)
ICH S11 (S9)
No
(4)
Identify factors to consider (s. Tab. 2)
(5)
Weight of evidence (WoE) review (s. Fig. 1)
(6)
WoE review warrants nonclinical safety testing
No
(6a)
Nonclinical safety testing is not needed
Yes
(6b)
Identified concern can be adressed without JAS
No
(6d)
Preliminary JAS/ DRF-study to assess tolerability is warranted
(6e)
DRF indicates tolerability for the intended paediatric population
Yes
(6c)
Concern should be adressed without JAS nonclinically and/or clinically through risk mitigation
Yes
(6f)
Definitive JAS is warranted (Timing)
No
(10)
New safety signals from ongoing and/or finished drug development steps [(4) = (6c), (6d), (6f), (7), (8), (9)]
(11)
Changes Of
-Age ranges
-Route of Administration
-Treatment duration
-Product indication
-Product formulation
(12)
WoE review should be revisited (5)
(7)
Further nonclinical studies (e.g., PPND)
(8)
Clinical studies (adult and/or paediatric)
(9)
Side effects of (approved) drugs of the same class/ World of science
ICH M3, E11, S5, ...

Flowchart: ICH S11-driven decision process to verify the need of additional nonclinical studies in support of the development of paediatric medicines. First of all, the investigational new drug (IND) intended for paediatric use should be assigned according to its class (1-3) to the appropriate guideline (scope) [7; 14]: Vaccines, gene and cellular therapies as well as tissue products generally do not fall within ICH S11′ scope (2); anti-cancer drugs (3) fall within ICH S11′ scope regarding the implementation strategies ('HOW'), for the question if additional nonclinical testing is needed ICH S9 should be applied; all other drugs are within ICH S11′ scope. For INDs which fall (completely) within ICH S11′ scope a Weight of Evidence (WoE) review (5) should be applied to address the question whether additional safety testing is needed ('IF'). The appropriate use of the WoE review requires the identification of all relevant factors (4). The overall decision process provisionally ends with a negative WoE vote (6a); but if additional nonclinical studies are warranted (6), a WoE-based determination of the test strategy is crucial (6b-f). If possible, the identified concern should be addressed without (6b) juvenile animal studies (JAS). If JAS are warranted, preliminary JAS in the form of a dose range finding (DRF) study is strongly recommended (6d+e). If the DRF study does not indicate the necessary tolerability, the WoE review should be revisited; otherwise, definitive JAS are (6f) warranted. New safety signals (10) from ongoing or finished drug development steps (particular safety signal from clinical studies) and approved drugs in use are a reason for the reassessment (WoE review) of the investigational drugs safety profile (11). This also applies for changes in age ranges, route of administration, treatment duration, drug product formulation and/or indications (12). The WoE review (5) should be conducted when designing the initial paediatric development plan [7; 11; 14–16]. (Based on ICH S11)

1.3 Aim Of The Present Thesis

The aim of this heuristic master thesis is a critical review of ICH S11' guidance on nonclinical safety testing and their consequences for the overall development of paediatric medicines.

For this purpose, the following ICH S11 issues should be mainly considered:

- The Scope
- The new *Weight of Evidence* Review (i.e., a decision-making tool for determining the need of additional nonclinical safety studies)
- The value of alternative testing approaches to JAS
- The approach to dose range finding studies
- The consequences and value of definitive JAS
- The timing of JAS
- The approach to the paediatric-first/only development

A full discussion of the choice of species and the design of nonclinical safety studies lies beyond the scope of this thesis.

Beside the ICH S11 guideline the following documents are of particular interest: complementary guidelines within the ICH-landscape, guidelines, reports and databases from competent authorities and scientific peer review publications.

2 RESULTS AND DISCUSSION

To fulfil the outlined objectives above, relevant ICH S11 core messages had been worked out, and supplemented by further sources, and were placed in the context of both the nonclinical and overall development of paediatric medicines. On this basis an interim conclusion has been drawn (for reasons of clarity towards the final conclusion) and, where appropriate, an alternative to the ICH S11 recommendations is suggested.

2.1 ICH S11' Scope – A Hat Not For Every Head

(see Flowchart (1-3))

The overarching goal of the ICH is the international harmonisation of regulatory requirements to ensure the preferable broad provision of high-quality medicines in the most resource-efficient manner. Generally, the tool of choice to achieve this goal is the implementation of ICH guidelines to the entire drug development process [17].

The proclaimed goal of the guideline ICH S11 consists in setting international standards for the nonclinical safety evaluation of pharmaceuticals intended for the paediatric use [7]. Under which conditions the ICH S11 recommendations could be applied is written down in its section *Scope*. According to the scope there are three aspects essential to consider (see Table 1): Firstly, the kind of medicine (e.g., vaccine, gene therapy) or indication (e.g., anticancer); Secondly, whether a nonclinical safety study (IF) should be conducted; and thirdly, if necessary, how to design it (HOW).

Kind of Medicine/ Indication	IF	HOW
Tissue engineered products, gene and cellular therapies, and vaccines	Potential S11	?
Anticancer-pharmaceuticals (S9)	S9	S11
Small molecule therapeutics and biotechnology-derived pharmaceuticals (S6)	S11	S11
All the other pharmaceuticals	S11	S11

Table 1: Scope of the ICH S11. The scope depends on the drug (i.e., Kind of Medicine/ Indication) and whether (IF) and how (HOW) to conduct additional nonclinical safety studies for the development of paediatric medicines [7]. (Based on ICH S11)

As it can be seen from the table above, ICH S11 scope does not cover all kinds of pharmaceuticals. For instance, to support the nonclinical development of paediatric anticancer drugs two ICH S-guidelines are of significant importance: ICH S9 guideline to address the question, whether (IF) to perform additional nonclinical studies and, if so, ICH S11 for the guidance of such studies (HOW) [7; 14]. The answer to the question whether (IF) to conduct additional nonclinical investigations could be derived from the following ICH S11 statement: "The conduct of additional nonclinical investigations should be undertaken only when previous nonclinical and human data are judged to be insufficient to support paediatric studies" [7]. In comparison the instruction in ICH S9' section *Nonclinical Studies to Support Trials in Pediatric Populations* reads as follows, "the conduct of nonclinical studies […] should be considered only when human safety data and previous animal studies are considered insufficient for a safety evaluation in the intended pediatric age group" [14]. Interestingly, although the ICH S11 refer to ICH S9 regarding the question whether to perform additional studies a significant divergence in the ratio of both requirements is not visible. In the case of tissue engineered products, gene and cellular therapies, and vaccines arises following from ICH S11: they are "excluded from the scope […] because dedicated […] studies are generally not warranted for

such products. However, some of the thinking outlined in this document about evaluating safety with existing information can apply" [7]. In other words, to "HOW" or whether (IF) to conduct nonclinical studies for such products is ICH S11 to its own statement not at all or possibly partly applicable. For this restriction ICH S11 delivers neither a reference to another ICH guideline nor a ratio. Consequently, ICH S11' "some of the thinking" statement to whether to perform nonclinical studies necessarily rises the following question: What of the thinking can and cannot apply for such products, and why? And what is the scientific ratio behind this statement? Therefore, answers to these questions are also therefore exciting as ICH S11 restriction appears contradictory to the universal useful WoE approach (see Section 2.2). A clarification of these questions would enable, if necessary, a derivation of action. Furthermore, the use of the terminology anticancer pharmaceuticals, small molecule therapeutics and biotechnology-derived pharmaceuticals as well as tissue engineered products, gene and cellular therapies and vaccines does not appear coherently and raises an admittedly heretical and nevertheless exemplary question: Does a *Chimeric Antigen Receptor* (CAR) T-cell anticancer therapy fall within the scope of the ICH S9 anticancer or a gen and cell-based guideline?

Interestingly, the ICH safety guidelines S11 and S5 (*Detection Of Reproductive And Developmental Toxicity For Human Pharmaceuticals*) show differences in pharmaceuticals to be included, although both guidance's covers major intersection of the ontogenetic development [15]. More precisely, the ICH S11 applies only partially to anticancer drugs and vaccines, in contrast to the ICH S5 (R3) which was issued two months earlier. At the same time, both guidelines give guidance to same developmental periods: From *birth to weaning* and from *weaning to sexual maturity* (i.e., stage E+F of Developmental and Reproductive Toxicity (DART) testing) [15].

In conclusion, 20 years after the implementation of ICH E11 for the clinical investigation of medicinal products in the paediatric population ICH S11 fills the nonclinical gap within ICH landscape in favour of the overall development of

medicines for children [18]. Furthermore, the ICH S11 provides in depth guidance to the nonclinical part of development programs that are tailored to paediatric specific indications (see Section 2.7). Unfortunately, ICH S11'scope does not apply to all pharmaceuticals, the reasoning behind that remains unclear. Based on ICH S11 used terminology a clear allocation of a pharmaceutical to one nonclinical safety guideline is not in any case possible.

2.2 ICH S11' Weight Of Evidence Review - The Template-Established Common Sense

(see Flowchart (4-6))

Before ICH S11 was adopted by the Regulatory Members of the ICH Assembly three regional guidelines on juvenile animal testing had been in force that address, in varying degrees, the need for such studies [9; 12; 13]. These conflicting recommendations and the missing further instructions particularly facilitated the conduct of similar animal studies between regulatory regions without substantial added value and hampered a preferably quick and wide availability of medicines for children. Even the ICH M3 *Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* only states that the conduct of any juvenile animal toxicity studies "should be considered only when previous animal data and human safety data, including effects from other drugs of the pharmacological class, are judged to be insufficient to support pediatric studies" [11]. However, the ICH M3 does not provide any criteria for this complex decision-making process. Therefore, the implementation of ICH S11 *Guidance on Nonclinical Safety Testing in Support of Development of Pediatric Medicines* presents an attempt to **"**provide clarity in determining the situations where non-clinical safety studies are important to support pediatric development" [19]. This undertaking should succeed with its core piece: the Weight of Evidence (WoE) review. Generally,

the WoE review is "used to describe the type of consideration made in a situation where there is uncertainty, and which is used to ascertain whether the evidence or information supporting one side of a cause or argument is greater than that supporting the other side" [20]. The ICH S11 delivers quality criteria for this method (see Section 2.2) as well as case studies as examples for the evidence-based weighting (see ICH S11 *Appendix B*) that should result (in the ideal case) in a clear, consistent and harmonised approach within and across all regulatory regions [7].

Subsequently, ICH S11 WoE review are presented in consideration of thesis objectives (see Section 1.3). Therefore, ICH S11 recommendations were supplemented by further sources and alternative proposals where appropriate.

2.2.1 The identification of factors - What has to be to considered?

(see Flowchart (4))

The identification and assessment of potential safety concerns should precede by the compilation and design of the nonclinical safety programme, especially for pharmaceuticals intended for paediatric use [7]. For this purpose, a broader set of information must be considered including the paediatric clinical development plan for the pharmaceutical discussed [16]. A single factor could not be seen sufficient to evaluate safety concerns in the intended paediatric population. The ICH S11-based WoE approach brings together and summarises information that includes the clinical context (pharmacology, pharmacokinetic/ absorption, distribution, metabolism, and excretion (ADME)) as well as the nonclinical (*in vitro* and *in vivo* animal) and clinical safety data (adult and/or paediatric) (see Table 2).

	Clinical Information	Pharmacological properties	Pharmacokinetic data	Nonclinical safety data
Youngest Intended Age	●			
Effects on Developing Organ System		●		●
Amount/Type of Existing Data	●		●	●
Pharmacological Target has a Role in Organ Development		●		
Selectivity and Specific*ity* of Pharmaceutical		●		
Clinical Treatment Duration	●			

Table 2: Compilation of factors to be considered for the application of the WoE review [7]. (Based on ICH S11)

Thereby, the amount and type of available information depend essentially on the kind/ timing of the overall drug development process. For instance, in comparison to the traditional paediatric drug development using deferrals to move step-by-step from the adolescent to younger age groups, the paediatric-first/ only development almost always requires additional nonclinical safety studies (see Section 2.7). In any case, it is essential for the WoE review to be taken into consideration for any paediatric population as the outcome can be different for each trial (see Flowchart (11)).

According to ICH S11, the following WoE factors are importante to determine the need of additional nonclinical safety investigations:

a. The Youngest Intended Patient Age

   The younger the youngest patient age in the intended clinical trial, the more likely additional nonclinical studies are warranted, thus supporting the safety in such patients. Even though, classifications of children in age groups are arbitrary to a certain extent. The stratification in subpopulations should preferably be based on developmental biology.

b. Effects on Developing Organ Systems

   Findings can give rise to concern for paediatric use particularly if effects occur on developing organ systems. Therefore, any nonclinical data that potentially indicate unwanted effects on growing tissues should be evaluated. Furthermore, findings are listed below that warrant additional nonclinical safety investigations:

   - Findings occuring in organ/tissues that undergo a critial ontogenetic development at the intended paediatric age, particulary in animals at similar exposures as those likeley to be achieved in clinical trial participants
   - Safety signals in adult animals in more than one species
   - Toxicity in adult animals that did not result in unwanted side effects in adult humans, but occurs in developing target organs/systems in the intended paediatric age

c. Amount/Type of Existing Data

   To a certain extend not only the content but also the type of existing data determines if additional safety investigations are warranted. In this context, clinical safety data particular of other paediatric subgroups are most likely

seen as the most valuable source. The ADME of administered medications are substantially affected by the age-depending maturation process of underliying organs such as the gastrointestinal, liver and renal system; resulting in increased importance of consideration of pharmacokinetic data. This could result in differing efficacy and safety profiles in neonates and infants compared to the adult human. Furthermore, the following points are regarded as useful to contribute to the WoE review:

- Results of genotoxicity and safety investigations usually supporting adult clinical trials
- Findings of reproductive and developmental (i.e., pre- and postnatal development (PPND)) studies (if available and biologically relevant)
- Safety signals in adult animals in more than one species
- Data from JAS already conducted
- Findings derived from clinical pharmacology and modelling and simulation tools that could complement existing data

d. Pharmacological Target has a Role In Organ Development

The fairly well visible ontogeny of organs underlies an ontogenetic development on the molecular level. This developmental expression pattern of pharmacological targets (e.g., receptors, enzymes, ion channels and proteins) could affect the sensitivity of developing organs. Therefore, the role of the pharmacological target should be understood, particulary for organs under development. In this context, concerns for paediatic use warrant additional nonclinical studies.

e. Selectivity and Specificity of Pharmaceuticals

In general, the higher the selectivity (i.e., receptor-bonding properties) and specificity (i.e., organ-bonding properties) of the pharmaceutical of interest the more predictable is its effect on the test subject (provided that the role of the pharmaceutical target is also known). Pharmaceuticals with lower selectivity or specificity for their pharmacological target have a higher probability of causing side effects. The listed factors (see subitems b, d, e) need to be considered when evaluating the pharmacological properties of the investigational drug. Further nonclinical investigations should be considered when the pharmacology of an pharmaceutical has a potential impact on the organ development or is not understood.

f. Clinical Treatment Duration

The duration of clinical treatment is another factor that should be considered in determing the need of further nonclinical studies. The probability to affect a paediatric subject during a developmentally sensitive window increases with the length of treatment and regularly warrants additional safety studies to the same extent. Nevertheless, "even a short-term exposure can have deleterious effects if it occurs at a vulnerable time of organ development" [7].

The ICH points out that the WoE tool as it is depicted in the ICH S11 is not all-inclusive for every situation, further specific factors such as risk mitigation must be considered if necessary (see Flowchart (6b,c) and Section 2.3). The final decision whether additional safety studies are warranted should include the translatability (biological relevance) and technical as well as practical feasibility of the intended test system and study design. Particularly in the case of JAS dose range finding studies are advisable to assess the tolerability prior to definitive JAS (see Flowchart (6e) and Section 2.4).

2.2.2 The weighting of factors – A balancing act

(see Flowchart (5))

After all relevant factors have been identified for evaluating the need of additional safety studies (see Flowchart (4), and previous Section) they are required to be weighed collectively and against each other according to ICH S11 WoE review (see Figure 1). Thereby, both factors the single factors *youngest intended patient age* and adverse *effects on developing organ systems* in particular, are needed to be weighted stronger than the others. In ICH S11' *Appendix B* several case studies are listed which show how to apply the WoE approach. Basically, from these cases it emerges that additional nonclinical studies are regularly warranted if at least one of the first two or more than one of the following conditions are met:

- the youngest intended patient age is younger than two years
- adverse effects on developing organ system are known or could potentially occur

- there are no or sole nonclinical safety data (in general, clinical data, particularly form other paediatric subgroups are the most valuable ones to assess the safety prior to clinical trials)
- the pharmacological target (could) has a role in organ development
- selectivity and specificity of the pharmaceutical is low or unknown
- the pharmaceutical is intended for chronic use

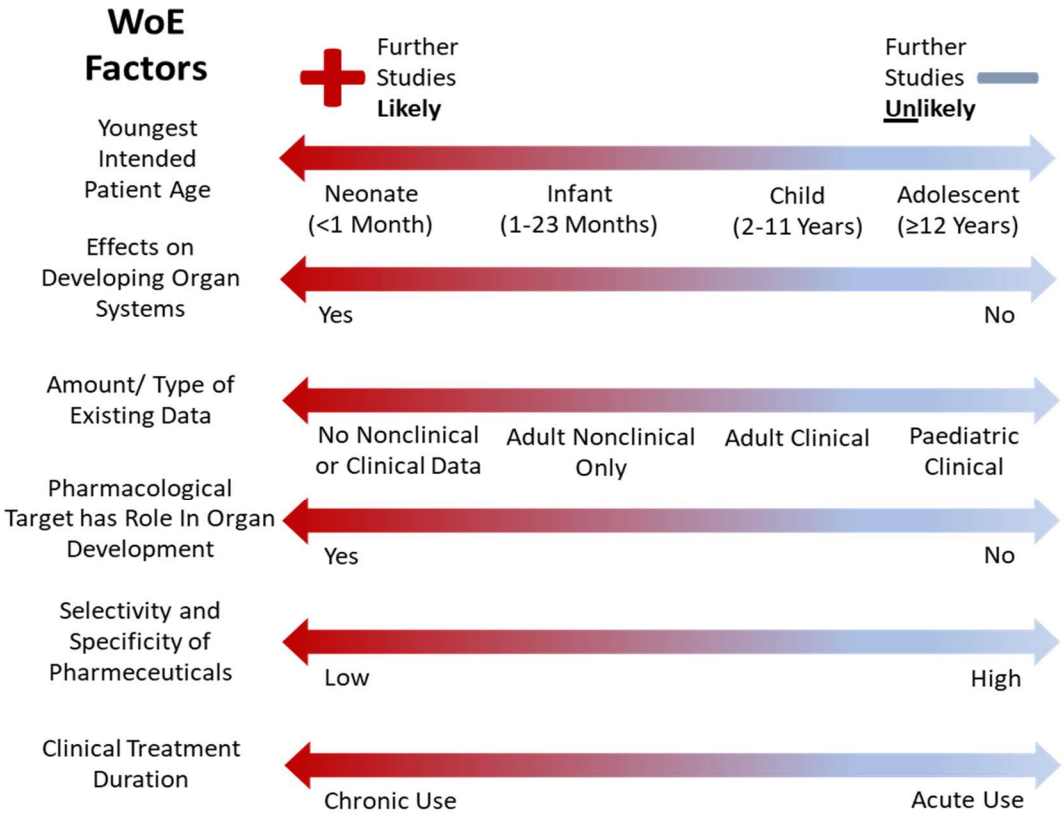


Figure 1: The WoE approach. The key WoE factors (left column) should be considered as depicted (arrows) to determine the need of additional nonclinical safety studies. The most important factors (*Youngest Intended Patient Age* and *Effects on Developing Organ System*) need to be weighted stronger than the following factors (not listed in order of importance) [7]. (Source: ICH S11)

According to ICH S11 own statement the *youngest intended patient age* is one of the most important factors to be considered. The weighting of this factor takes place gradually according to ICH E11 four paediatric subsets: pre-term and term neonates (0–27 days), infants (1–23 months), children (2–11 years) and adolescents (12–18 years) [7]. Thereby, intended clinical trials in pre-terms and neonates more likely justify additional non-clinical studies; the lowest probability for further safety investigations exists for the adolescents.

Age Categories	**EMA** (partly WHO based)	**FDA**	**ICH** (E11/S11)
Neonates	0 - 2 years	0 – 1 month	0 -27 days
Infants		1 month – 2 years	28 days – 23 months
Children	Pre-Schoolers: 2 - 5 years	2 -12 years	2 to 11 years
	Schoolers: 6 - 9 years		
Adolescents	10 - 18 years	12- 16 years	12 to 16 -18 years*

Table 3: Paediatric age categories according to EMA/WHO, FDA and ICH [7; 16; 21; 22]. (Based on EMA, FDA, ICH)

At first glance (see Table 4)., numbers of JAS in all paediatric investigation plan (PIP) per age category (agreed by December 2015) underpinning this kind of weighting in practice: At least one JAS in support of clinical investigation in children ≤ 2 years of age was performed in 167 of 232 (71%) PIPs; whereas the remaining 65 PIPs containing at least one JAS (29 %) were intended in support of clinical trials with children above the age of two [5].

Therapeutic area	Number of PIPs (by Dec 2015)	Number of PIPs with at least one JAS (% of all PIPs in therapeutic area)	Number of PIPs for children ≤ 2 years of age, with at least one JAS (% of all PIPs in therapeutic area)	Number of PIPs for children > 2 years of age, with at least one JAS (% of all PIPs in therapeutic area)
Total	**881**	**232 (26%)**	**167 (71%)**	**65 (29%)**
Psychiatry	**17**	**10 (59%)**	**1 (10%)**	**9 (90%)**

Table 4: Overview of PIPs containing at least on JAS; by therapeutic area and age range (children below and above 2 years of age) [5]. (Source: EMA)

However, any classification of the paediatric population into age remains to some extent arbitrary categories (see Table 3), as the differing organ-systems of each individual develops and mature nonlinear in stages (see Figure 2). Attention should also be also paid to the fulfilment of developmental milestones that could differ

considerably between cultural regions such as the introduction of solid food and weaning. The EMA guideline *Ethical considerations for clinical trials on medicinal products conducted with minors* has further sub-divided the age group 2–18 years into pre-schoolers (2–5 years), schoolers (6–9 years) and adolescents (10–18 years) [21]. The relative wide age span of the latter age group triggers the ongoing discussion of an appropriate subdivision of the paediatric population which ideally reflects a separation of all relevant developmental milestones: a subdivision of the *World Health Organisation' (*WHO) defined age group of the adolescent starting by the age of 10 has already been demanded [23]. All these examples including the age classification of the U.S. Food and Drug Administration (FDA) illustrate impressively that age groups can be subdivided differently (see Table 4), "and often these categories are only used to provide guidance for regulatory and clinical reasons but do not reflect the maturity of the individuals", which is generally recognised as a crucial aspect that has to be taken into account during the conduction of paediatric clinical trials [23].

	Critical period of structural and functional growth and development
	Active period of growth and/or functional maturation
	Slow continued growth and/or refinement of function
	Structurally and functionally fully mature

Figure 2: Categorisation of different phases of structural and functional development [7]. (Source: ICH S11)

Nevertheless, the ICH S11 points out that "decision on how to stratify by age should focus on developmental biology" [7]. For this purpose, ICH S11 delivers an overview (see ICH S11 *Appendix A*) that illustrates the age dependent development of human organs systems (see ICH S11 Table A1) and the commonly used toxicology species

rat, beagle dog, Göttingen minipig and cynomolgus monkey (see ICH S11 Table A2-5).

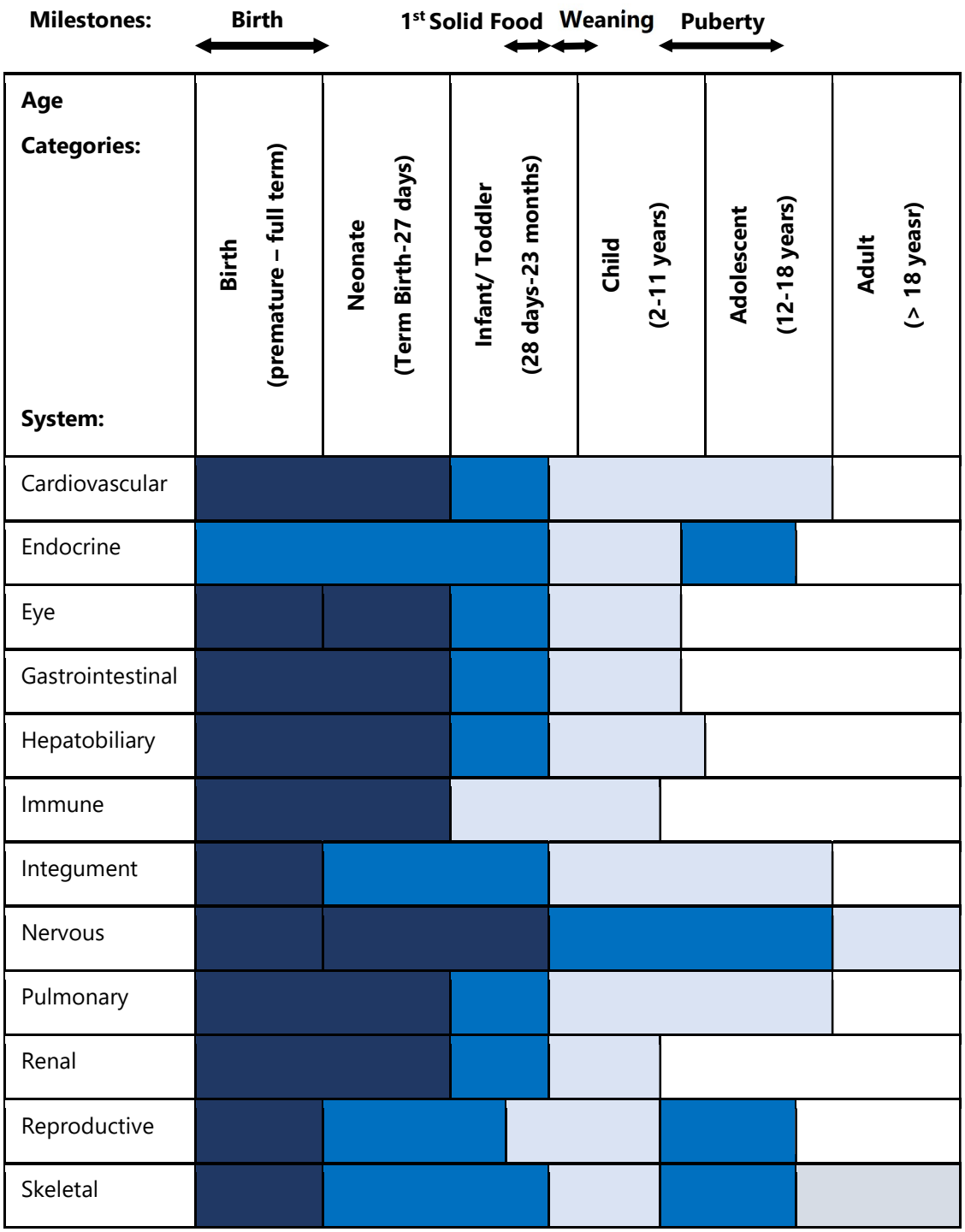


Figure 3: Schematic representation of age-dependent development of human organ system. The color coding shows the level of maturity depending on organ system, paediatric subgroup and reached milestones (i.e., birth, 1st solid food, weaning, puberty) [7]. (Based on ICH S11)

This overview illustrates in depth, compared to already existing and highly regarded paediatric nonclinical safety guidelines [9; 12; 13], interspecies similarities and differences in the organ/system development with the help of four for the toxicological assessment relevant development phases (see Figure 2). Essentially, the sequential presentation of species-specific timing and duration of single development steps should allow a standardised "assessment of the relevance of existing nonclinical data, as well as the selection of species, starting age, and dosing duration for a JAS" [7].

It is crucial that attention is paid during critical and active periods of functional and structural growth (see Figure 2, fist two rows (blue coloured)). Empirically, developmental toxicity is of particular concern during these two dynamic periods "and less so during periods of slow growth or refinement of function" [7]. In humans the active developmental period of several organ systems can last until 18 months of age (see Figure 3).

The active developmental period of several organ systems until 18 months of age explains the implementation of the *youngest intended patient age* within the WoE review as a potential safety concern which increases gradually with declining age. However, it is a matter of fact that active development periods for certain organ-systems occur regularly up to adulthood, examples include the skeletal and the reproductive system, i.e., puberty, a period of intense endocrine activity. Particularly, the development of the highly complex central nervous system (CNS) which comprises the establishment of, e.g., pain pathways, myelination, and cognitive functions in different timelines up to the age of 18 years raise concern regarding the administration of drugs in clinical trials with adolescents [24]. Also, a closer consideration on *EMAs 10-year report on the experience acquired as a result of the application of the Paediatric Regulation* (see Table 4) reveals that 90% of PIPs with at least one JAS in the therapeutic area of psychiatry relate to children above the age of two [5]. In contrast, the total average of all PIPs with at least one JAS in the age

groups above 2 years is significantly lower (29%). A survey performed by the Centre for Drug Evaluation and Research' (CDER) *Division of Neurology Products* to JAS intended for the treatment of CNS-related diseases shows positive findings in 72% (18/25) of all JAS that correlates to children ≥ 2 years of age at the initiation of dosing [25]. *Van der Laan et al.* (2019) analysed nonclinical studies to 15 CNS-compounds for the treatment of, e.g., attention deficit hyperactivity disorder (ADHD), depression, epilepsy, and schizophrenia up to adulthood [26]. The results of 12 JAS revealed more severe toxicities and evidenced novel CNS effects compared to their adult counterparts. On this basis, the children, and adolescents in the therapeutic area of psychiatry could be identified as a particular vulnerable subpopulation [25; 26]. However, a final evaluation of age-depending effects of CNS-compounds requires a deeper analysis, that includes among others the disclosure of the rationale that warrants these JAS including their outcome in detail, the covered developmental period and indication. Regardless of these emerging questions, many experimental studies on behavioural toxicity in juvenile animals identify rather the peripubertal development age as another critical developmental period, with increased risk for developmental toxicity. For example, *Bolanos et al.* (2003) showed that the administration of methylphenidate (MPH is used for the treatment of ADHD) during pre- and peri-adolescent development of rats results in a significant higher sensitivity to stressful situations, increased anxiety-like behaviours, and had enhanced plasma levels of corticosterone in the long-term [27]. Further studies where MPH and clomipramine (antidepressant) has been administered to adolescents caused behavioural changes, i.e., depression-like signs, that endure into adulthood [28; 29]. The studies mentioned for the treatment of CNS-related diseases reveal similar pattern of drug response that depend on maturational stage of exposure (see Figure 4). Essentially, maladaptive drug responses occur relatively frequently and considerably after the peri-adolescent treatment period of CNS-related diseases. Also, the ICH S11 states that "functional maturation occurs into adulthood in humans for some aspects of CNS development

"and point further out that they "cannot be fully modelled by animal test systems" [7]. *Van der Laan et al.* conclude that "detailed clinical observation and motor activity measures were the most powerful end points to detect juvenile CNS effects" [26].

According to ICH S11, the *youngest intended patient age* for clinical treatment is one of the most important factors to determine whether further studies are warranted. More precisely, the probability for further nonclinical studies decreases gradually with increasing age. This tenet is depicted in ICH S11' WoE tool in form of an age-related scale that neglects the potentially vulnerable age group of adolescents. This approach seems to be questionable, as experimental studies on behavioural toxicity in developing animals indeed identify the adolescence as another critical developmental period, which results in an increased risk for adverse drug effects [27–31]. Instead, the risk assessment should rather take into account the relative age of development of each organ system of relevance (see Figure 5).

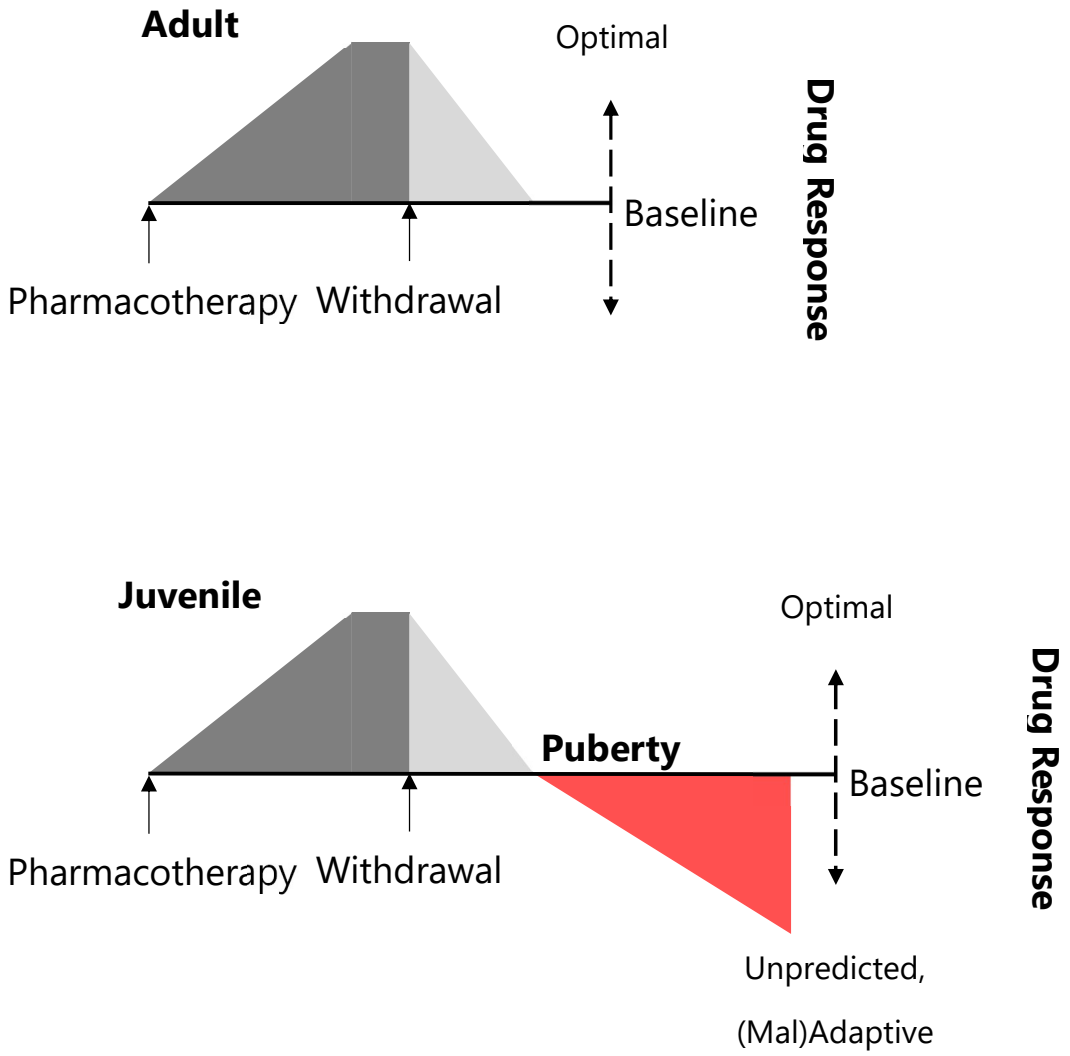


Figure 4: Trajectories of drug response depend on the maturational stage of exposure. A post pubertal (adult) pharmacotherapy (top) leads ideally to adaptive drug responses (left), whereas a per-pubertal (juvenile) drug exposure may (or may not) also produce symptom improvements (bottom). Upon withdrawal (right), the trajectory of drug response progresses after puberty in form of an over-compensatory maladaptation (e.g., depression triggered by antidepressant) [30; 31]. (Based on *Andersen et al.*)

According to ICH S11, the *duration of clinical treatment* is another factor that should be considered when making the decision whether additional nonclinical studies are warranted. The longer the duration of treatment (e.g., 3-months, 6-months, chronic intermittent), the more likely additional safety studies are warranted. This tenet is based on two assumptions that a longer drug treatment leads to appropriate higher

exposure and that a longer drug treatment could affect more likely a sensitive developmental window. And this in turn could increase the probability of unwanted side effects in the intended population. Even though the WoE factor discussed corresponds to already existing recommendations [9; 13], these two assumptions are only conditionally correct. The exposure is essentially determined by the drug (e.g., composition, bounding properties and dosing) and degree of maturity of the pharmacokinetic/ ADME underlying organs/systems. However, the maturity of pharmacokinetic/ ADME underlying organs/systems and the drug properties are concerns that are already addressed by other WoE factors (e.g., *Youngest Intended Patient Age* and *Selectivity and Specificity of Pharmaceuticals*). Much more difficult is the assumption that a longer drug treatment could more likely affect a sensitive developmental window. Finally, an exception to the rule can have serious consequences. The ICH S11 states that "even a short-term exposure can have deleterious effects if it occurs at a vulnerable time of organ development" and provides actual by itself why the WoE factor *duration of clinical treatment* should not be used to determine the necessity of additional safety studies (at least in form of a linear based assessment). Consequently, the gradually illustration and of the WoE factor *duration of clinical treatment)* is flawed as it images a general probability and hampers a single case consideration (just like the illustrated WoE factor *youngest intended patient age)*.

The other WoE factors (effects on developing organ dystems, type/ amount of existing data, pharmacological target has a role in organ development, selectivity, and specificity of pharmaceuticals) are not considered as critical for the evaluation whether additional safety studies are warranted and therefore not discussed due to practical constrains.

Together with the WoE review, the ICH implemented an innovate decision tool to evaluate whether additional nonclinical safety studies are warranted. It provides clarification within and between different regulatory regions and simplifies the quite

complex process of the integrated risk assessment. The risk assessment requires a deep knowledge of the organ development of both, the human and the test species commonly used (see ICH S11 *Appendix A*). For this purpose, the ICH S11 delivers a high-level overview of organ development by species that necessarily surpasses by far appropriate illustrations in already existing paediatric nonclinical safety guidelines [9; 12; 13]. Furthermore, a probably standardised application of the challenging WoE review by different users also requires several application examples; unlike to ICH S8' (Immunotoxicity studies for human pharmaceuticals) WoE review, this methodical quality criterion has been fulfilled by 4 case studies in ICH S11' *Appendix B* [7; 32].

However, the gradual weighting of the single factors *youngest intended patient age* and *duration of clinical treatment* within the WoE illustration conflicts with scientific findings [30], ICH S11 own overview to the age-dependent organ development (e.g., progression of the CNS-system up to adulthood comes along with a potentially increased vulnerability) and hints to the sensitive window of organ development (ICH S11: "even a short-term exposure can have deleterious effects") [7]. More precisely, the present illustration of both WoE-factors is flawed and in turn could result in a flawed risk assessment with its entire consequences for the intended paediatric population. Even if numerous specialists (e.g., paediatricians, pharmacologists, statisticians and specialists from regulatory affairs, nonclinical pharmacology (pharmacodynamics) and safety (especially toxicology and pharmacokinetics)) and relevant institution (e.g., non-clinical working group (NcWg) and paediatric committee (PDCO)) supervising the nonclinical development plan for a paediatric pharmaceutical, the WoE review should be adapted to ensure a priori the probably best convergence to the clinical situation. A proposal (see Figure 5) suggests the replacement of WoE top scale *youngest intended patient age* by another one called *organs/systems of relevance in development during/after treatment*. In comparison to the WoE-factor *youngest intended patient age* the new WoE-factor explicitly consider the organ system of relevance including its relative

degree of maturity. The tenet here is: The less the degree of maturity of the organs/systems of relevance during/ after treatment, the more additional safety investigations are warranted. This approach should sharpen the focus on the organ-ontogenies of relevance for the intended drug including the development period after treatment (i.e., off-treatment period). Nevertheless, the assessment of potential off-target effects caused by systemic exposure during the dynamic development of systems important for ADME (i.e., gastrointestinal, liver, and renal systems) remains challenging. These effects occurring predominantly in neonates and infants, i.e., in subpopulations where ADME underlying organs undergoing an intense development. Therefore, nonclinical safety studies are almost always justified for these subpopulations (independent of the predictability of the intended nonclinical studies). However, based on ICH S11 own statement that "JAS is not informative in predicting and recapitulating age-related difference in ADME" the use of JAS is questionable in assessing systemic exposure as result of age-depending change of the ADME performance. Off-target effects which are predominantly caused by an insufficient selectivity and specificity should be sufficiently covered by the WoE-factor *selectivity and specificity of pharmaceuticals*. A quantification of the weighting factor *organs/systems of relevance in development during/after treatment* could be standardised by an adapted figure which depicts the relative degree auf maturation of each organ system depending on age. Ideally, a scoring system can be implemented that include the four relevant phases (see Figure 2) for the toxicological assessment with the most vulnerable point/period of all relevant organs/ systems between the start and end of treatment as well as the further development of the appropriate organ-system (possibly, based on as open-source tool). Also, this alternative approach could be better suited to reveal potential toxicities on developing organs following a short time exposure. In this case, the WoE-factor *duration of treatment* should be deleted entirely.

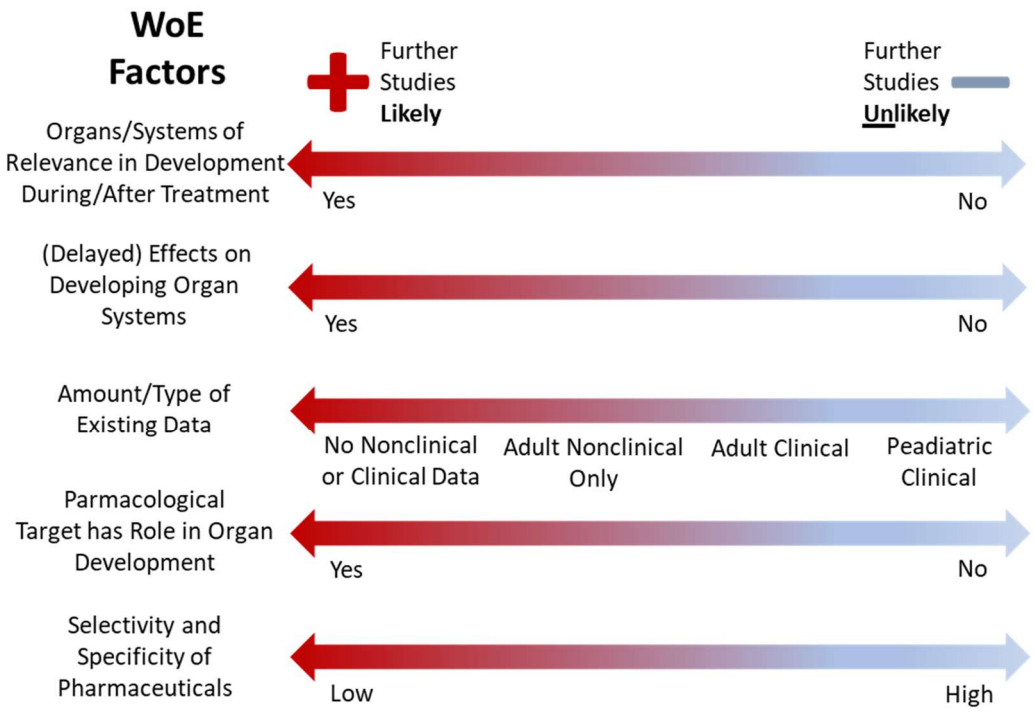


Figure 5: The alternative WoE approach. Within the alternative WoE approach the factors *Organs/Systems of relevance in Development During/After Treatment* and *(Delayed) Effects on Developing Organ System* are most important. They should be weighted stronger than the following factors (not listed in order of importance) [7]. (Based on ICH S11)

2.2.3 The Outcome – The pros and cons regarding additional nonclinical safety studies

(see Flowchart (6a))

After all relevant factors have been identified (see Flowchart (4) and Section 2.2.1), the WoE approach should be used to determine whether additional nonclinical investigations are warranted. The ICH S11 emphasised that the clinical risk assessment relies on including "the totality of the evidence" or rather "multiple factors", "a single factor should not be considered in isolation". Thereby, the focus will essentially be on the factors considered most important to inform the decision-making process ("Examples of applying the WoE approach are in *Appendix B*") [7]. It must be taken into account that the outcome of the WoE review can be different for different applications of the same pharmaceutical. A revision of the WoE review is indicated in case of changes to the paediatric age, route of administration, treatment duration, drug product formulations and/or indications (see Flowchart (11)). However, ICH S11 provides a set of simple single factor driven cases where further nonclinical safety investigations are warranted or not (see Table 5) [7]. Identified safety concerns and the intended clinical use determines the specifics to be evaluated and these in turn the kind (e.g., in vitro, in vivo (see Section 2.3)), design (not subject of this thesis) and time (i.e., before or in parallel to clinical trial phase 2b (see Section 2.6)) of additional nonclinical investigations. In any case, "the study objectives should be aligned with the WoE outcome and the intended paediatric use."

Nonclinical Safety Guideline ICH S11 [7]
When additional safety studies (or rather JAS) are <u>not</u> warranted (see Flowchart (6a))
JAS is not informative in predicting and recapitulating age-related differences in ADME
If adult clinical data are available and exposure is not occurring at a vulnerable time of organ development, a JAS is not considered important to support initiation of short-term PK studies in paediatric patients
JAS is not warranted to confirm toxicity in target organs in which sensitivity to toxicity is not expected to differ between adults and paediatric patients
Further nonclinical studies might not add value when the existing pharmacology information has already identified a particular hazard unless a more detailed understanding of the dose response relationship or differences in sensitivity between adult and juvenile animals is warranted
Additional nonclinical studies are not warranted when existing clinical safety data and risk mitigation strategies are considered sufficient to support paediatric use
When additional safety studies (or rather JAS) are warranted
Differences in target or off-target tissue development are a concern that should be considered
Findings occurring in animals at similar exposures as those likely to be achieved in paediatric subjects are of increased concern, particularly if the findings occur in organs/tissues that undergo critical postnatal development at the intended paediatric age
If […] the role of the pharmacology on development is not understood or not reasonably predictable, further nonclinical investigations should be considered

Table 5: Compilation of single cases simplify the decision-making process to additional nonclinical studies [7]. (Based on ICH S11)

Interestingly, the ICH guidance insists on the consideration of "the totality of the evidence" and consequently on the implementation of the WoE review [6]. On the other hand, besides its case examples in *Appendix B*, ICH S11 listed several relatively simple single cases in its main text whether additional nonclinical safety studies are warranted or not. The ICH S11 guidance differentiate from the EU and US guidance in almost all counts regarding the extent and depth of information, particularly to its core piece: the WoE tool and its supplementary *Appendix A* (*Overview Of Age-Dependent Development Of Organ Systems By Species*) and *B (Case Studies Applying the Weight Of Evidence Approach)* [2; 3; 6]. In comparison, in the EU and US guidance "special risk/benefit assessment" and "risk-benefit analysis" are used without a sufficient definition that should include case examples ensuring a probably standardised approach. Both regional guidelines are remarkably similar, however, the European one specifies rather the circumstances when a case-by-case approach, i.e., WoE review, should be applied. Examples include products under development for "specific paediatric indications or life-threatening or serious diseases without current effective therapies" [6]. Furthermore, the EU guidance essentially restricted itself to cases where JAS would be informative or necessary [3]. The US guidance also provides clear examples where JAS might not be warranted. This is the case when "(1) data from similar therapeutics in a class have identified a particular hazard and additional data are unlikely to change this perspective; (2) there are adequate clinical data and adverse events of concern have not been observed during clinical use; (3) target organ toxicity would not be expected to differ in sensitivity between adult and pediatric patients because the target organ of toxicity is functionally mature in the intended pediatric population and younger children with the functionally immature tissue are not expected to receive the drug" [2].

In conclusion, to achieve an outcome consistent with the regulatory requirements whether nonclinical studies are warranted the ICH S11 insist on the use of "the totally of evidence", i.e., the application of the WoE review. In principle, this approach could

be seen as the scientifically only correct method. However, the US, EU and even ICH-guidance provides in different extent a set of relatively simple single cases where additional nonclinical safety studies are warranted (or not). Furthermore, it is a fact that a result of sufficient magnitude in only one important factor *(organ/system of relevance in development during/after treatment (previously youngest intended patient age)* and *(delayed) effect on developing organ systems)* should trigger additional nonclinical safety investigations, and this regardless of other WoE-factors (unless further studies are not expected to change the perspective). Based on this insight, a compilation of single factors of sufficient magnitude and clear and common cases would help to further standardise the decision-making process. Within the decision-making process, such a compilation (with no guarantee) could be placed in front of WoE-review (see Flowchart (between step 4 and 5) as part of an alternative work approach. In case of further uncertainties, the WoE-tool could be used to ascertain whether the evidence warrants further nonclinical investigations or not.

2.3 Additional Nonclinical Safety Studies – JAS or Something Else?

(see Flowchart (6 b-f))

Different approaches are conceivable to deal with an identified safety concern regarding the paediatric population. In fact, not each identified safety concern warrants *per se* further measures such as additional nonclinical safety studies in juvenile animals. Therefore, ICH S11 mentioned different strategies supporting clinical studies in the intended paediatric subpopulation [7]. Following approaches could be used alone or in combination instead of or beside JAS:

I. modified pre- and postnatal developmental (PPND) and repeated-dose toxicity (RDT) studies

II. alternative methods to JAS (*in vitro* and *ex vivo* investigations as well as *in silico* methods)

III. clinical risk mitigation strategies (not part of this thesis)

In this context, it should be noted that additional nonclinical safety studies are only justified if the study objectives are in alignment with the WoE outcome and the intended clinical use. The selected approach must be biologically relevant (i.e., of translational significance), feasible (i.e., practical, technical and at sufficient systemic exposures) and as far as possible consistent with the 3Rs principle (reduce, refine, replace principle with regard to animal experiments) [33].

In the ICH S11 to varying extent and depth approaches mentioned above (see point I. and II.) were discussed in the following in alignment with the objectives of this thesis (see Section 1.3). Due to practical constraints, this thesis cannot provide a comprehensive review of clinical risk mitigation strategies (see point III. above) in paediatric trials and the standard design of JAS.

I. <u>Modified PPND and Repeated-Dose Toxicity Studies</u>

To streamline the paediatric drug development process is an early consideration of the supporting nonclinical part crucial. As rising doubts about children's safety participating in clinical trials could be potentially addressed during the nonclinical development plan primary considering the adult patient. Therefore, according to ICH S11 a change of the design and/or timing of the traditional nonclinical program should be considered [7]. This approach is in accordance with the corresponding US- and EU-guidance and comprises essentially the modification

of two types of studies: the pre- and postnatal development (PPND) as well as the repeated-dose toxicity (RDT) study [9; 13]. Even though PPND studies encompass the period from the implantation to sexual maturity (i.e., stages C to F of development and reproduction toxicity (DART)), the focus of DART testing is primarily on development before birth, "with only limited assessment of postnatal developmental effects" [15]. Whereas RTD studies are mainly conducted in peripubertal animals (e.g., rats ≥5 weeks or dogs ≥6 months of age) [34]. Aim of modified toxicity studies is to close the gap between both the standard PPND toxicity study and toxicity studies in peripubertal animals [35; 36]. Depending on the age-related dosing scheme is a modification of one or even both study types conceivable. The modified PPND (mPPND) study is especially characterised by a timely deviation (e.g., initiated just before the PIP-application (EU), see Y-axis of Figure 6) from the normal drug development paradigm (see ICH M3) and some endpoint-modifications which include the offspring [11; 15]. A modified RDT (mRDT) could be designed to allow an initiation of dosing at younger age "to support the corresponding developmental stages in paediatric patients" (see x-axis of Figure 6) [7].

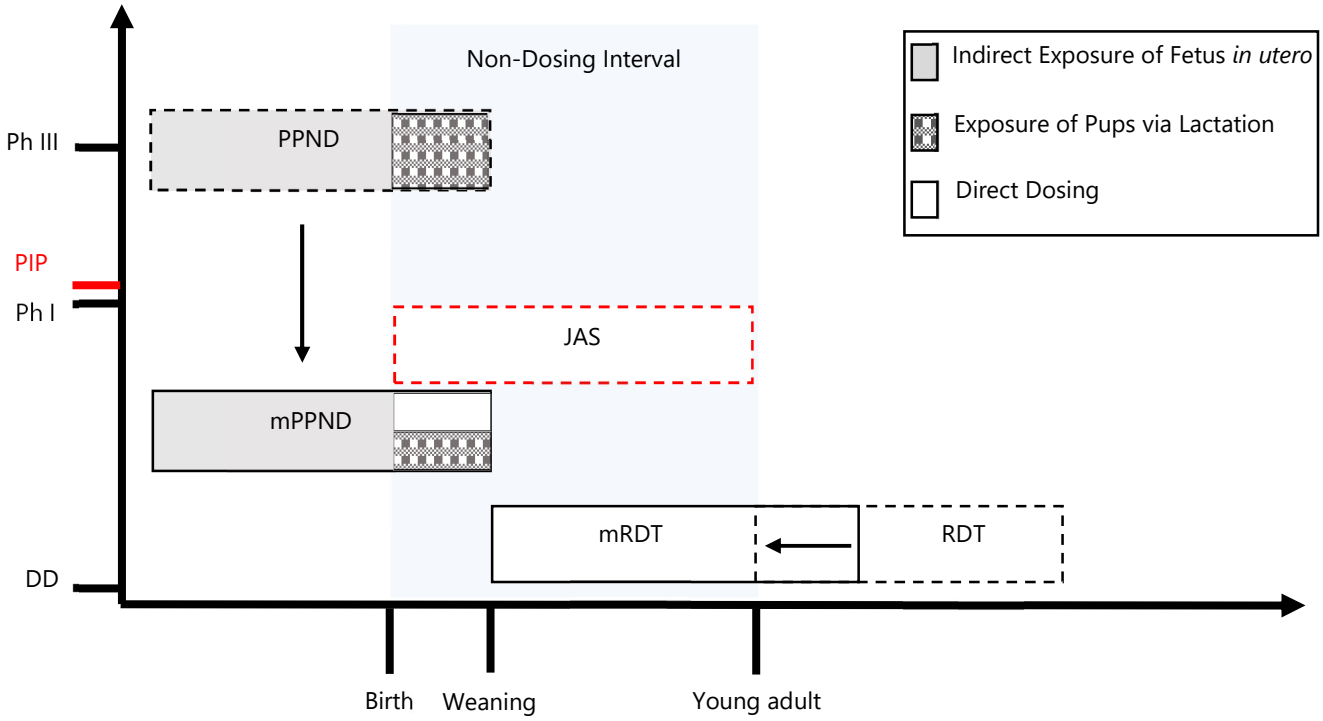


Figure 6: Modifications of the traditional nonclinical program to assess toxicity in developmental stage of concern. To implement the findings in the paediatric investigation plan (PIP (EU)) the pre- and postnatal developmental toxicity study (PPND framed dashed) can be shifted form the third (Ph III) to the first clinical phase (Ph I). The final modification of the PPND (modified PPND: mPPND, framed continuous) requires additional endpoints and, if necessary, dosing could be changed (direct dosing if practical) and extended towards the non-dosing interval (grey background usually covered by juvenile animal studies (JAS)). Similarly, the approach to the repeated-dose toxicity study (RDT, framed dashed), the modified RDT (mRDT, framed continuously) can be initiated, as usual, after drug discovery (DD) [36]. (Based on *Mulberg et al.*)

According to ICH S11, "these changes can replace or refine the design of a JAS" [7]. The guideline also explains when mPPND studies are of relevance (e.g., at "clinically relevant systemic exposures in offspring during the postnatal period") and mentions typical pitfalls regarding the outcome-evaluation (e.g., "maternal and fetal tolerance of the drug should be considered because they could influence interpretation of the findings in offspring."). In contrast to the EU- and

US-guidance (e.g., histopathology, evaluation of suitable developmental parameters) further instructions for the design of an appropriate mPPND study are lacking. Even though the safety assessment can be streamlined by a mPPND study in terms of time and number of juvenile animals used for the whole drug development, a set of challenges should be considered [37]:

a. The addition of further endpoints to the standard PPND study design could affect the technical/practical feasibility
b. Exposing the pups directly instead via the maternal milk could lead to exaggerated toxicity due to the changed drug exposure and pharmacokinetic (PK) profile
c. As ICH S11 points out, adverse effects could be ascribed especially to the exposure *in utero* and are not necessarily of translational significance for the intended subgroup
d. In few cases only, the therapeutic drug exposure can be achieved for technical reasons. For example, oral bioavailability in juvenile animals is often too low for drugs intended for intravenous treatment

II. Alternative Methods to JAS *(In vitro*, *ex vivo*, and *in silico* methods)

The ICH S11 points out that "when a study is deemed warranted, […] the nonclinical investigation […] could be a JAS or another study (e.g., *in vitro* or *ex vivo* investigations)" [7]. However, the ICH S11 delivers solely one further guidance to "another studies" (i.e., alternative testing methods). This guidance reads as follows: "*in vitro* or *ex vivo* investigations using juvenile [i.e., animal] or paediatric [i.e., human] tissues or matrices (e.g., serum, urine) might be useful to determine potential age-related differences in sensitivity" [7].

In the context of *Step 3* of the formal ICH procedure [38] the *International Council on Animal Protection in Pharmaceutical Programs* (ICAPPP) criticises the shortcoming of nonclinical testing methods besides JAS [39]. Therefore, the

ICAPPP demanded the implementation of a further section that "provide guidance on the use of *in vitro* and *ex vivo* methods to support the development of paediatric medicines." This part should be prefixed to the already existing section "Design of nonclinical juvenile animal studies" and considered preferentially. The main argument for the demanded paradigm shift is the limited significance of JAS for the paediatric population (see Section 2.5) and according to ICPAAA the availability of "relevant and important" methods that could be used instead of JAS. In the comments to the draft ICH S11 guideline the ICPAAA refers to three publications that deals with testing strategies that could replace JAS; these methods include essentially the use *of in vitro* methods/ organoids (*Berkers et al*., 2019), adult clinical data (*Visalli et al.*, 2018) and *in silico* methods (*Smits et al*., 2018) [40–42].

In one of these publications *Berkers et al.* demonstrated that drug effects correlate between the used *in vitro* model, i.e., patient-derived stem cells (organoids) and *in vivo*; here, clinical response of patient with cystic fibrosis [40]. The *in vitro* findings showed a significant correlation with the lung response of patients with a treatment-depending median age of 15, 16 and 35 years. A regimen was administered one or two times, different effects between the single and the multiple dosing were not detectable.

Keeping in mind the organogenesis of the lung (functional development up to 24 months of age followed by a period of slow growth up to adulthood where the alveolar surface increases) and that developmental toxicity is the alterations of the developmental processes rather than functional alterations of already developed systems the presented *in vitro* model could not be considered as a tool to address paediatric-specific safety concerns. However, the findings are promising regarding a more personalised and cost-effective drug development, but they are based on endpoints that are tailored to the benefit of the treatment, a kind of safety assessment was not performed.

Furthermore, IPACCC demanding for a new section with alternative testing strategies to JAS is also based on *Visalli et al*. findings [41]. The working group demonstrates that safe starting doses in paediatric oncology patients could be determined without any knowledge of findings in JAS but by solely using a fraction of the adult dose that is close 1. *Visalli et al.* reviewed the paediatric clinical data for 25 small chemical entities and 4 biologic anticancer therapeutics. The results of all 29 investigated drugs showed that the starting dose that is almost equivalent to the one used for the adult patients was safe in terms of life threating toxicities. On the other hand, dose limiting toxicities (DLTs)/adverse events (AE) were not detectable with standard monitoring plans for paediatric oncology trials as well as standard JAS (if carried out). These paediatric-only AEs (i.e., GGT, CPK, blast cell count, hyperuricemia) must be considered in the context of the administered anti-cancer drugs vismodegib, ruxolitinib, sunitinib and midostaurin. However, JAS data are only publically available for midostaurin, and the paediatric-only AE noted for this drug (i.e., increased blast cell count) was not observed in the performed standard JAS. Independent of the question, if a prior knowledge of these potential paediatric-only findings would have an impact on the clinical monitoring plans it is undeniable that a tailored JAS, as in the ICH S11 explicitly demanded, could potentially reveal these kinds of AEs in advance of the appropriate paediatric trials. In EMAs project report *Results of juvenile animal studies (JAS) and impact on anti-cancer medicine development and use in children* findings of JAS in 20 PIPs to anti-cancer medicine were compared to adult data [43]. The adult and juvenile study findings (non-specific effects on development landmarks and reproductive organs are excluded) revealed new target organ toxicities and increased severity of toxicity for 8 products each with tangible consequences for PIP, Summary of product characteristics (SmPC), risk management plan and use in children. Regardless of the final value of these animal findings (see Section 2.5), paediatric-only specific findings cannot be uncovered based on adult data.

Finally, based on the publication of *Smith et al.* the IPACCC demand the implementation of physiologically based pharmacokinetic (PBPK) modelling as a part of a standard default in lieu of JAS. *Smith et al.* are of the opinion that the use of the regulatory accepted PBPK modelling should be extended to the neonatal population [42]. At the same time, the workgroup recognises the poor predictive performance of the intended *in silico* method (see Figure 7): "for neonatal drug development, this tool is not yet sufficiently accurate…"

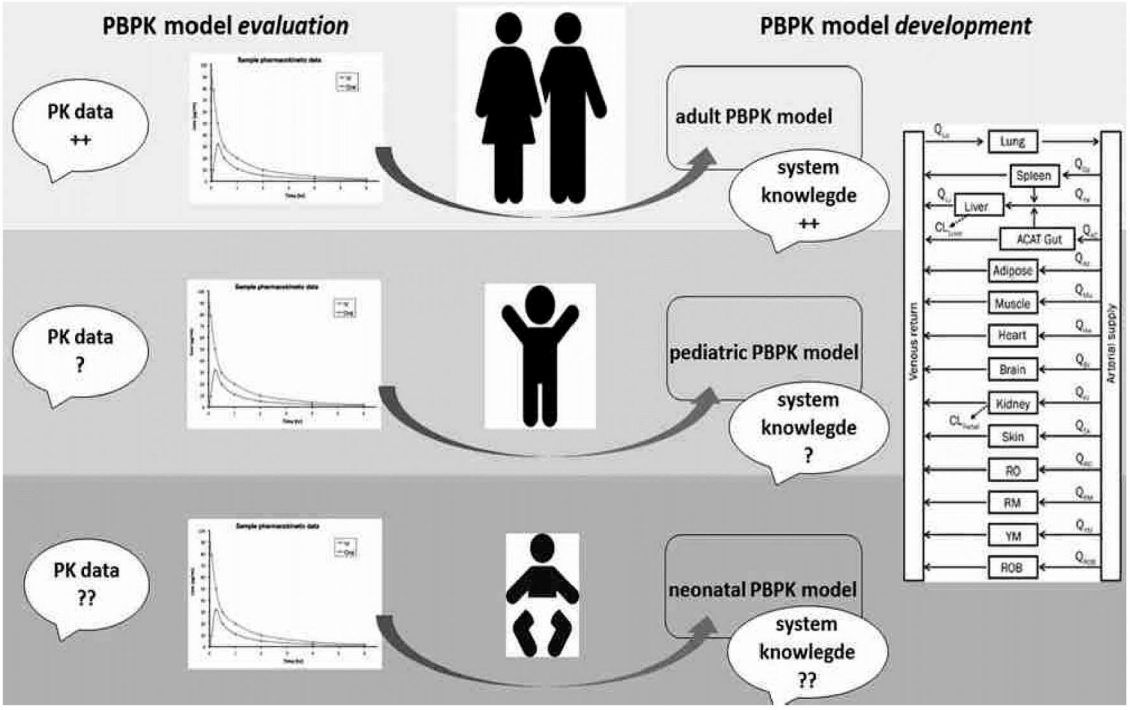


Figure 7: Schematic representation of the development and validation-status of age-dependent physiologically based pharmacokinetic (PBPK) models. Accurate PBKA modelling requires pharmacokinetic (PK) datasets and the population specific system information. Up to now, regarding the paediatric and in particular for the neonatal population is this tool not yet sufficiently accurate [Q = blood flow] [42]. (Source: *Smith et al.*)

However, *Smith et al.* are convinced that this challenge can be met by the combination of the PBPK modelling with clinical observations. Current findings that are associated with the hepatic biliary excretion, renal tubular activity, and

central nervous system exposure showed that this combined approach could be sufficient to explain maturational and size-related changes to an extent that is almost impossible to reach by *in vivo* or *in vitro* data alone. Especially with regard to neonates, an extension of PBPK modelling considering the drug exposure by breastfeeding and hence the mother (postpartum) and newborn is desirable. Whereas the availability of crucial datasets on PK and maturational physiology is and remains a necessary perquisite for the refinement of PBPK model predictions. Therefore, a progress in the field of PBPK modeling and simulation in neonatal drug development requires the contribution of clinical researchers and willingness to data sharing. Source of hope is the Open Systems Pharmacology approach with its toolboxes PK-Sim® and MoBi® that enables, inter alia, fully transparent open-source development [44]. Moreover, the *Organisation for Economic Co-operation and Development* (OECD) offers the free *quantitative structure–activity relationship* (QSAR)-toolbox for *in silico* toxicity estimations [45]. Regulatory authorities have already been accepted *in silico* methods if OECD requirements on the validation are met. However, even this method has its inherent limitations, the PBPK modeling do not cover all required toxicity endpoints such as the *No Observed Adverse Effect Level* (NOAEL) [46].

Independent of IPACCC claims as discussed above, several efforts have already been made on the EU level to foster alternatives to animal testing. As mandated by the directive 2010/63/EU, one of these efforts was the foundation of the *European Union Reference Laboratory for Alternatives to Animal Testing* (EURL ECVAM) by the commission's *Joint Research Centre* (JRC) [47]. EURL ECVAM duties includes the coordination and promotion of the development and use of alternative methods; the "coordination of the validation of alternative approaches at EU level; and setting up, maintaining, and managing public databases and information systems on alternative approaches and their state of development" [48].

The EMA defines criteria for regulatory acceptance of alternative testing methods in its *guideline on the principles of regulatory acceptance of 3Rs (replacement, reduction, refinement) testing approaches* [33]. The guideline states that "data generated by using alternative testing approaches can be submitted in parallel to data obtained by using accepted testing methods on a voluntary basis" and continous that "the data achieved by 3Rs testing methods will not be considered for the regulatory decision but will be evaluated independently regarding the regulatory acceptance of this testing method in the future". With this aim in mind, the EURL ECVAM regularly publishes information on the validation status and regulatory acceptance of alternative testing approaches on its website.

The following information on alternative testing methods that could be potentially considered to estimate developmental toxicity was obtained from the EURL ECVAM *Database on Alternative Methods to Animal Experimentation* (DB-ALM) (accessed November 2021) and from the *EURL ECVAM status report* (2020) [49; 50]. The methods presented *to EURL ECVAM* for validation, identified in *ad hoc* reviews of the literature, and end-users in specific application areas are the main focus of the database. By 2019, 31 of 370 DB-ALM entries (i.e., alternative testing methods) are made that can be assigned to the topic area of developmental toxicity (see Appendix of this thesis). All these listed methods could be categorised - according to ICH S5 used terminology to *in vivo* studies - as segment I (fertility and reproductive performance) and/or segment II (teratogenicity and embryotoxicity) experiments. To this thesis, the validation-status of entries covering essentially the developmental period before birth has not been examined further. The proposed methods are mostly based on embryonic or placental cells/tissues of different origins. One exception that covers the pre- and postnatal period (segment III) to a sufficient degree is the *Extended One Generation Reproductive Toxicity Study* [51]. The alternative method is characterised by an extended exposure and observation of animals of the first generation of progeny up to postnatal day (PND) 70. As described and evaluated

in the OECD Test Guideline 443 the aim of this approach is the detection of developmental effects arising until sexual maturity [52]. However, the proposed method listed in the DB-ALM is similar to a modified PPND study (see point I. above). Also, alternative testing approaches that have the potential to replace JAS are not content of EURL ECVAM most recent Status Report (2020) [50]. Even a review of project results from EU-ToxRisk - a consortium of 39 institutions funded by the EU to implement new insights in alternative testing methods – issued between 2016 and 2021 does not deliver animal-free approaches primarily considering the effects of pharmaceuticals on the developing organism after birth [53].

However, to date, promising progresses in the field of alternative testing methods have been made. These includes highly innovative *in vitro* techniques such as organoids, "omics" and microphysiological systems (MPS) [54]. However, the development of accurate alternative testing methods is still challenging, particularly for complex endpoints such as developmental toxicity. None of the methods mentioned above has been validated by the EURL ECVAM for paediatric purposes and therefore their implementation into the ICH S11 is questionable. For the validation of computational approaches the EURL ECVAM has no mandate – the validation lies in the responsibility of the applicant [55].

Nevertheless, a promising stand-alone-approach in the long term instead of JAS has been outlined by *Marx et al.* (see Figure 8) [54].

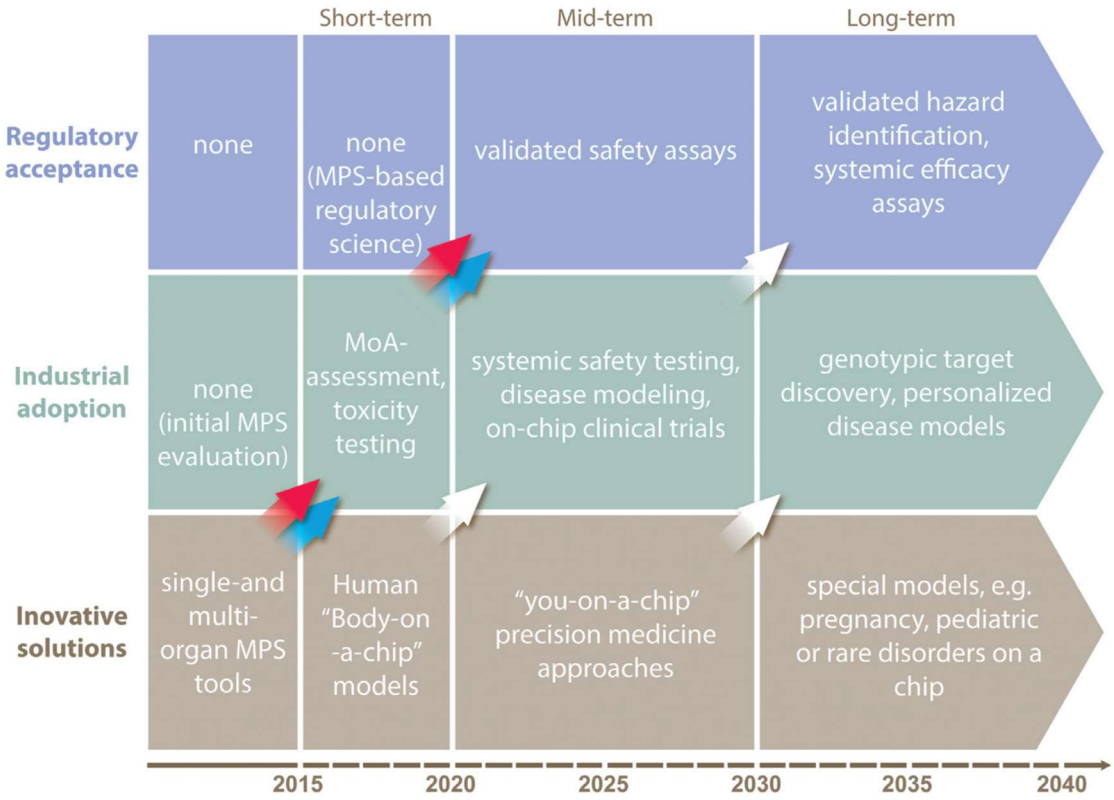


Figure 8: Time schedule towards the reduction and replacement of animals by MPS-based approaches.
The MPS-development includes three consecutive phases: innovative solutions (bottom row), industrial adoption (middle row) and regulatory acceptance (top row). Each development-step impacts the appropriate consecutive phase above as depicted by red diagonal arrows for single-organ, blue diagonal arrows for multi-organ and white diagonal arrows for human-on-a-chip MPS-based approaches [MoA = Mode of Action] [54]. (Source: *Marx et al.*)

The microphysiological systems (MPS) technology also known as "organs-on-a-chip" are in vitro models, refering to a tissue or small funcional unit of an organ. The composition of the funcional unit is similar to real organs/tissues and represent the normal physiology [54]. As of now, the developing "human-on-a-chip", indicates a systemic model representing the major organs in a single MPS which is meant to be utilised in such early clinical drug development. This will catalyse the formation of both efficacy and safety data, which usually is obtained

through clinical phase I, clinical phase II trials and systemic toxicity studies in animals [56].

Consequently, the Authors of the *Think Tank of Transatlantic Toxicology* are of the opinion, that the implementation of MPS-drug technology with its expected high predictivity into innovative development programs lead towards the reduction and replacement of animal and even human testing (see Figure 8) [54]. This would change the drug development paradigm from a step-by-step approach to an "ultrafast discovery and validation approach with an enormous economic impact and value for society", which includes the most vulnerable - the neonates and children (see Figure 9) [54].

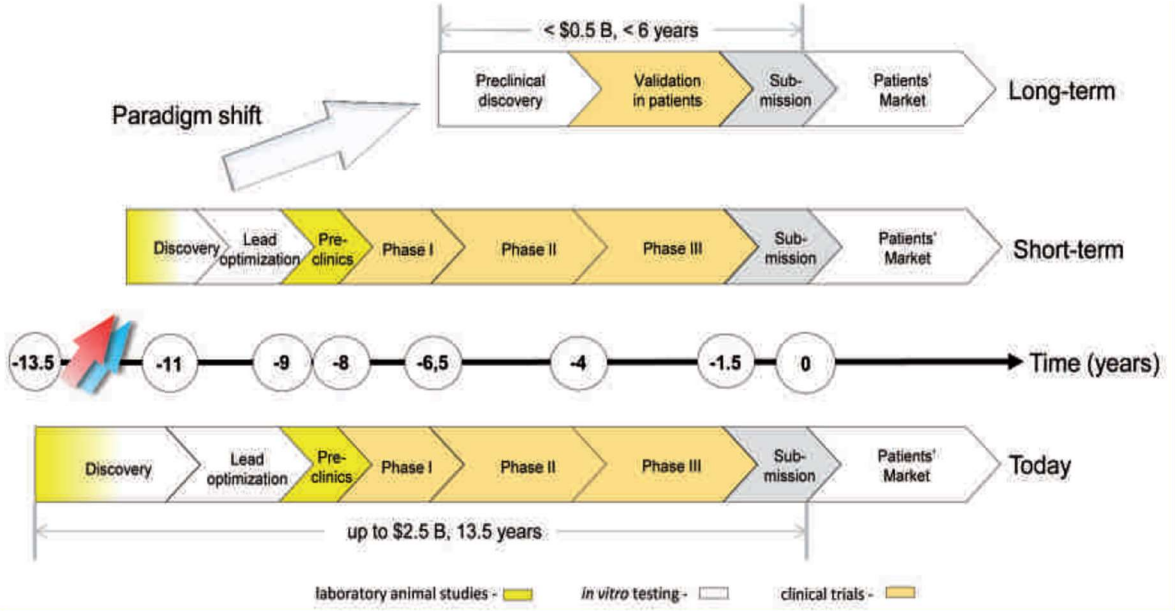


Figure 9: MPS-based change of the current drug development paradigm. The MPS-technology could have a direct influence on the current drug development (Today); initially (Short-term) through single- and multi-organ MPS approaches (red and blue diagonal arrows) in terms of time and cost, and finally through human-on-a-chip MPS-based approaches (white diagonal arrow) leading in a paradigm shift (Long-term) [54]. (Source: *Marx et al.*)

In conclusion, to deal with identified safety concerns the ICH S11 mentions several possibilities (see point I.- III. Above) beside JAS in varying degree. Regarding mPPND studies no further instruction was made which could complement already existing guidance [9; 13; 15]. Thereby, further instructions are needed to meet typical challenges such as the direct exposure of juvenile animals to the drug in another way than via maternal milk.

During the ICH S11 creation process, the IPACCC demanded the implementation of a section with alternative testing strategies in lieu of JAS as the preferred default approach. Based on IPACCC mentioned references and the EURL *ECVAM Database on Alternative Methods to Animal Experimentation* no validated approach could have been identified which could replace JAS.

For the validation of *in silico* tools such as the PBPK modeling the EURL EVCAM has no mandate – it lies in the responsibility of the applicant (e.g., pharmaceutical industry). The current system knowledge of paediatric/ neonatal PBPK modeling is limited but increasing. The data hungry approach of PBPK modelling still requires extensive datasets considering the special requirements of the paediatric population. To ensure this clinical researcher have to make their contributions by implementing a broad and particularly accurate application of the development related PBPK approach.

One promising approach that even surpasses JAS regarding its predictivity for the paediatric population could be the MPS or "organs on the chip" technology. In the long term, this highly innovative approach is intended to depict special models e.g., rare or paediatric disorders. As a consequence, a full developed and regulatory accepted MPS-approach significant changes of the paediatric drug development could be expected. These changes comprise a reduce and replacement of animal studies and even reduce of human testing, and this in turn will streamline the whole drug development process, including those with children. Finally, the effectiveness of the entire drug development process is

going to increase in terms of both time and cost (see Figure 8): The whole drug development process can possibly be accelerated twice as much (up to 6 instead 13.5 years) and the average investment in research & development could be reduced up to 80% (0.5 B instead 2.5 B $).

In general, testing methods to developmental toxicity require a set of complex endpoints as well as an in-depth knowledge of drug-triggered adverse effects and their underlying mechanism [46]. To take up this challenge several efforts have been made on the EU-Level such as the establishment of EU-ToxRisk during Horizon 2020 (project results have been available since 2016). None of the published project results in alternative testing methods that could replace JAS as default approach (Accessed in September 2021). Also, in 186 *European Public Assessment Reports* (EPAR) of medicinal products for human use (issued between July 2016 and July 2018) no alternative testing approaches have been identified that could replace JAS [57]. Conclusions to submitted registration dossiers published by the European Medicines Agency (EMA) via the centralised procedure do not show any indications to the use of alternative testing methods such as organoids (as requested by the IPACCC) and the MPS-technology [57]. Moreover, a further analysis that considers solely EPARs to drugs intended for the paediatric population including quantitative (i.e., number of alternative testing methods per EPAR) and qualitative (i.e., kind of testing method and proportion of sufficiently covered developmental toxicity endpoints) aspects would be desirable to estimate the use of alternative testing methods to JAS. However, based on the lacking entries in the DB-ALM to developmental toxicity can be assumed that the current practice is not sufficiently expedient regarding the implementation of a regulatory accepted and a more effective drug development approaches (see Figures 8 and 9). Part of this practice (beside the lasting strengthening of research efforts in the field of alternative testing approaches via EU-ToxRisk) is the possibility to submit data generated by alternative testing methods in parallel to data obtained by using regulatory accepted approaches on a voluntary basis. The

used alternative method intended for regularly regulatory testing should be formally validated by the *European Directorate for the Quality of Medicines* (EDQM) and finally EURL ECVAM [58].

However, the existing practice should be reconsidered due to the high significance of the nonclinical development for the entire paediatric drug development process (see Figure 9). More precisely, the significance for the paediatric population consists in the conjunction of the long, expensive and animal-based non-clinical safety assessment of quite doubtful predictability and the still adult-driven paediatric drug development and the accompanying neglected therapeutic needs in terms of not developed (not available) medicine for children (as pointed out by the *10-year Report to the European Commission - General Report on the experience acquired as a result of the application of the Paediatric Regulation* -) [5; 59]. The paediatric drug development is essentially driven by the adult drug development programme and that mainly for two reasons: firstly, the *paediatric regulation* obliges the applicant to submit a Paediatric Investigation Plan (PIP) towards the end of clinical phase I to drugs intended for adult use [1]; secondly, the development of adult medicines is still more attractive for economic reasons despite the implementation of monetary incentives for developed medicines for children. The main cost driver of the relatively complex paediatric drug development is the required subdivision of the paediatric population and the traditional drug development approach moving step-by-step from the elder to the youngest children. At the same time, the expected income for medicines for children is lower compared of medicines intended for the adult population. The reason for this is the composition and the expected development of the population (i.e., demography) in the rich and drug developing industrial world. In the *Paediatric regulation* implemented instruments of obligations and incentives are a success in terms of the numbers of developed medicines for children compared to the period before inception of the appropriate EU law [1]. However, in terms of the special need to be covered is the

paediatric regulation less effective [5; 59]. The paediatric regulation boosted the adult driven paediatric drug development even more. Moreover, in some cases the current practice absorbs essential resources because of the obliged conduction of clinical trials with children to indications with already existing and efficient drug options.

As a reaction to the challenges identified in the 10-year report to the paediatric regulation the EMA and European Commission implemented an action plan on paediatrics [60]. The action plan defines some measurable objectives to meet the identified challenges. But none of the defined objectives targets the nonclinical drug development to overcome the neglected development of paediatric medicines (i.e., neglected therapeutic needs; also because the 10-year report does not deliver a tailored analysis to used alternative testing approaches affecting the paediatric drug development as suggested above) [5].

Thereby, a streamlined non-clinical drug development using innovative technologies such as the MPS-based disease models could ultimately lead to the coverage of that neglected therapeutic needs. Technologies that significantly reduce the whole drug development cost as the MPS-technology intended to be could directly counteract the adult driven paediatric drug development. A significant reduction of the needed drug development investment increases the changes to cover the appropriate cost through the expected income of the finally launched medication, even for relatively small populations. This in turn could encourage the drug developers to invest in neglected therapeutic areas such as paediatric specific indications; similarly, as intended by the paediatric regulation with its obligation and incentives.

Moreover, a simplification of the nonclinical drug development process with the help of testing approaches that enables a much better predictability than JAS could reduce the required number of children in clinical trials. This alternative testing approach would also solve the recruitment challenge of several paediatric

drug development programs. At best, a few participants are needed to validate the highly predictive nonclinical result – with a sustainable steering role towards neglected therapeutic areas including rare paediatric diseases.

Due to the economic benefits, it also must be in the interest of drug developers to bring about a simplification of the whole drug development process (i.e., paradigm shift), as pointed out by *Marx et al.* (see Figure 9). However, to date, no alternative testing approaches have been regulatory accepted which could replace JAS. Overall, it can be concluded that alternative testing methods have the potential to impact the whole drug development process towards neglected therapeutic needs. On the other hand, it can be assumed that this challenge cannot been solved by an ICH-guideline. More convincible are aim-orientated approaches, away from the submission of data generated by alternative testing methods on a voluntary basis to an obliged approach in parallel to data obtained by using regulatory accepted methods. As this approach requires a broad research experience, databases and infrastructure, the suggested practice could be restricted to companies which would benefit (the more the R&D investmenst the more the savings potential) the most from a simplified drug development process. Due to the significant socio-economic impact and because we just love our kids, generous public founding of start-ups or rather companies with the core competency in the development of alternative approaches such as the MPS technology is more than justifed. Moreover, research efforts as conducted under EU-ToxRisk need to be strengthened further to gain a deepened knowledge of the underlying pathomechanisms of neglected diseases. As required for the development of highly innovative technologies such as MPS and to cope with idiopathic and rare paediatric diseases. By then, bridging technologies (e.g., CRISPR) could be used for the humanisation of already regulatorily accepted animal approaches such JAS (true the motto make the most of what you have), with the aim to increase the value (see Section 2.5) of JAS for the paediatric population [61].

2.4 The Juvenile Dose Ranging Approach - Trail and Error in Pure Form

(see Flowchart (6d,e))

Once additional safety investigations are warranted, the following points should be considered [7]:

- The aim of the nonclinical investigation ought to be compatible with the WoE outcome, i.e., identified concerns
- The nonclinical investigation should be technically and practically feasible regarding the experimental setup and endpoints
- And in the case of JAS, a dose range finding (DRF) study in juvenile animals usually recommended to increase the quality of the definitive JAS

The technical and practical feasibility of the intended experimental animal study is associated with the chosen test species. To assist the species selection, the ICH S11 provides a detailed overview to *Principal Advantages and Disadvantages of Various Mammalian Species for Use in Juvenile Animal Studies* (*Appendix A*: Table 6A) [7].

After a test species has been chosen a DRF study should be considered to confirm tolerability and adequate exposure. Dosages used in the adult toxicology studies are often not informative regarding the tolerated dose levels in juvenile animals [62]. A DRF study can help to identify differences in exposure between age ranges. Particularly, DRF studies in animals of the youngest age envisaged for definitive JAS are recommended to evaluate the usually most critical phase for tolerability and exposure differences. DRF studies are also useful to refine the study design of the definitive JAS, e.g., by the identification of further endpoints. However, the DRF study design is generally characterised by a limited number of (core)endpoints (e.g., pathology). Further features of a DRF study are a short duration and a reduced number of animals.

If differences in exposure between age rages occur, an adaption of the dosing regimen in the definitive JAS should be considered.

In the case of incomprehensible findings (e.g., greater sensitivity or significant differences in toxicity) the review of available information (e.g., ADME, safety, ontogenesis) can be instructive. Otherwise, a tailored investigative JAS could be warranted to narrow down the sensitive development period or gain knowledge about the underlying toxicity.

A persistent lack of tolerability at exposures as expected for paediatric patients could have significant implications for the corresponding age range. An intended alteration of the paediatric clinical age range must be accompanied by a total reassessment (i.e., WoE approach) of potential safety concerns (see Flowchart (11)).

In principle, findings from DRF studies are a basis for discussion with health authorities during the PIP submission. Finally, findings resulting from JAS may lead to PIP modifications (e.g., granting of waivers for studies in younger children), a more focused safety monitoring and entries in both SmPCs and EPARs [63].

Unlike a definitive JAS, juvenile DRF studies have not to be conducted according to Good Laboratory Practice.

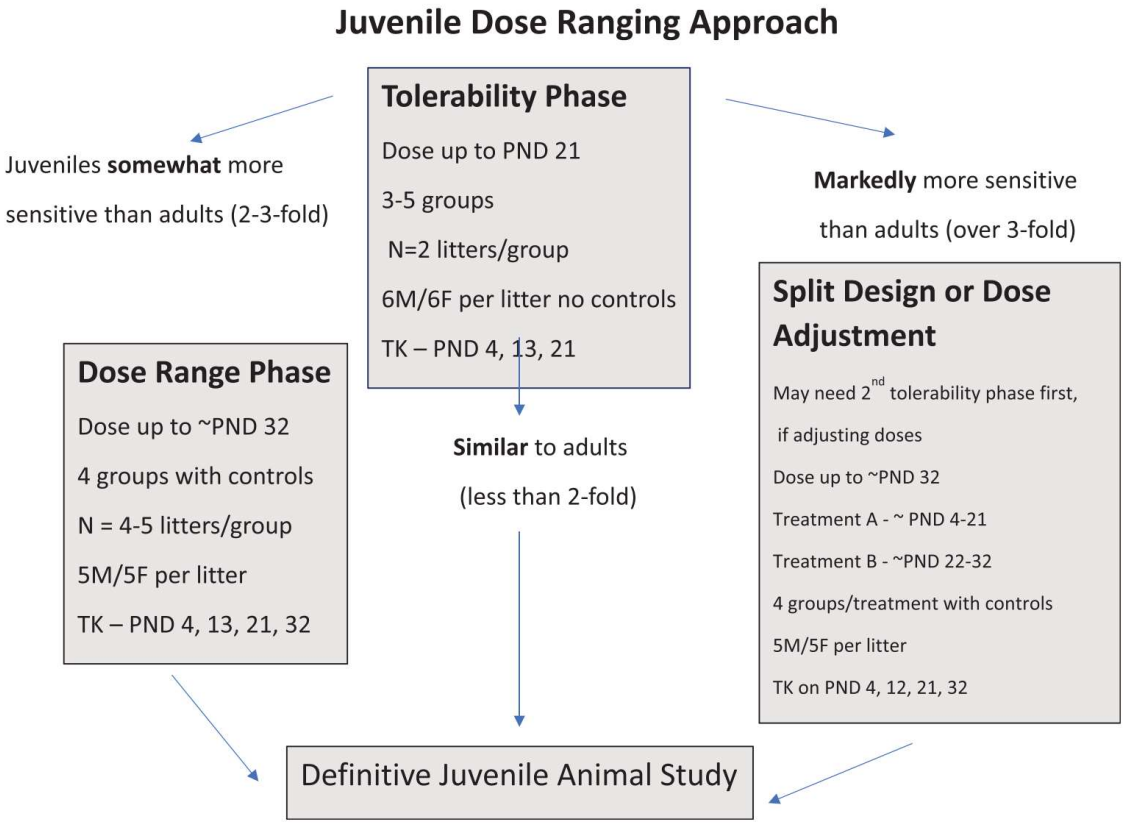


Figure 10: The dose ranging approach with preweaned rodent animals. A tolerability phase with relatively few animals is usually sufficient for determining potential differences in tolerability and exposure between adults and juveniles. Then, depending on the results three approaches are useful, either a dose range phase with or without dose adjustment (left box); a split design using two cohorts, one dosed in the preweaning and another dosed in the postweaning period (right box). In few cases, a progression straight to a definitive JAS (bottom box) is warranted [64]. (Scoure: *Posobiec et al.*).

Just like the ICH S11, a recent study emphasised the particular importance of DRF studies when initiation of dosing is intended in the early preweaning period [64]. The reason for this is the dramatically changing ADME of maturing animals during the conduct of the study. However, the ICH S11 do not provide a clear approach this challenge similar to the standard JAS (see ICH S11 *Appendix C*).

In contrast, *Posobiec et al.* established a default approach for dose ranging juvenile toxicity studies on the basis of 45 JAS. More precisely, the DRF approach is tailored

to definitive JAS studies where start of dosing is intended in the preweaning period. It consists of 2-3 phases (see Figure 10): First, a tolerability phase with small numbers of animals. Then, depending on the findings, the following could be performed: "a dose range study with or without dose adjustment, and adjustment to the JAS design, or in rare cases, progression straight to a JAS" [64]. This approach (i.e., when a juvenile tolerability/dose range study preceding definitive JAS) resulted in only 2 of 10 (20%) definitive JAS with "test article-related preweaning mortality" [64].

In conclusion, a well-planned and performed juvenile dose range study has direct implications on the quality of definitive JAS. As a rule, in terms of an increased tolerability and maintenance of consistent exposure to the drug over the study's critical development period. This in turn leads to meaningful data for the identification of safety concerns and extrapolation of risks to the paediatric population.

JAS are relatively often requested by health authorities in support of trials with children below the age of two (see Table 4), particularly to the age range corresponding to the preweaning period. However, despite of detailed explanations to DRF studies in general, the ICH S11 do not provide a clear dose range finding approach for studies with preweaned animals. Because of the particular importance for the qualitiy of definitive JAS and finally the interpretation of data for the corresponding paediatric population, an implementation of the phased DRF approach (see Figure 10) into the ICH S11 should at least considered.

2.5 Consequences And Value Of JAS – Still Questionable

(see Flowchart (6f))

2.5.1 Clinical and regulatory consequences of JAS findings

New safety signals (see Flowchart (10)) arising from JAS are a reason to inform the WoE review once again (see Flowchart 12)). Finally, findings of definitive JAS could have the following consequences [63]:

- PIP modifications (e.g., granting of partial and full waivers)
- Safety monitoring improvements
- Reports in SmPCs and/or EPARs (in some cases accompanied with warnings based on safety grounds)

By December 2015 (see Table 6), the PDCO granted 129 (out of 881 conditions (14 %)) waivers (i.e., waiving of clinical trials with certain paediatric subsets). In some cases, a PIP comprises in some cases more than one condition, i.e., more than one development programme. And the waived development programmes are based on safety reasons of different origins; for example, derived from JAS or other paediatric trials. JAS were part of 26% (232/881) of all review PIP conditions. These JAS contributed data to a significant extent to 39% (50) of all granted waivers (either full or partial). Whereby, 71% (165/232) PIPs conditions containing JAS were intended for children of two years of age or younger. And 71 of these PIPs included newborns until the twenty-eighth day of life (neonates).

The table below (Table 6) provides an exact list of full and patrial waivers based on safety reasons by therapeutic area and waiver age range (waiver based on JAS findings are displayed, too).

Waiving studies for younger age ranges based on safety grounds arisen in JAS could trigger the administration of medicines normally restricted to the elder children for a broader age range and outside the authorised indication. To address this concern, the implementation of JAS based safety reasons in the SmPC seems useful

(independent of the final value of JAS findings for the corresponding paediatric population).

			Waiver						
Therapeutic area	**Full waiver**	**Partial waiver**	**< 1m**	**< 6m**	**< 1y**	**< 2y**	**< 4y**	**< 6y**	**< 12y**
Anaesthesiology	-	2	-	-	-	2	-	-	-
Cardiovascular diseases	1	7 (4)	1	-	1 (1)	3 (2)	-	2 (1)	-
Dermatology	2 [1]	6	1	-	-	-	3	2	-
Endocrinology – gynaecology – fertility - metabolism	14 [6]	8 (3)	1	-	-	4 (2)	1	1	1 (1)
Gastroenterology - hepatology	3	9 (1)	-	3 (1)	-	1	5	-	-
Haematology - haemostaseology	-	3	-	2	1	-	-	-	-
Immunology – rheumatology - transplantation	7 (6)	5 (1)	-	-	-	2	2	-	1 (1)
Infectious diseases	2 (1)	15 (3)	3	2	-	1	2 (2)	4	3 (1)
Neurology	1	4 (1)	1	-	-	2	-	1 (1)	-
Oncology	1	9 (2)	1	4	2 (2)	2	-	-	-
Ophthalmology		2	-	-	-	-	-	1	1
Pain	6 (1) [1]	1	-	-	-	1	-	-	-
Pneumology - allergology	1 [1]	-	-	-	-	-	-	-	-
Psychiatry	4 [1]	2 (1)	-	-	-	-	-	2 (1)	-
Uro-nephrology	2 [1]	3	-	1	1	1	-	-	-
Vaccines	1	4	-	1	1	2	-	-	-
Other	1	3 (2)	-		1 (1)	-	-	1 (1)	1
Total	**46 (8) [11]**	**83 (18)**	**8**	**13 (1)**	**7 (4)**	**21 (4)**	**13 (2)**	**14 (4)**	**7 (3)**

Table 6: Number of JAS contributed to full and partial waivers. "Numbers in the table represent the total numbers of full and partial waivers based on safety grounds. Out of 881 conditions in total, 129 contained either full or partial waivers based on safety grounds. In case of (multiple) modifications, each PIP/ condition was only counted once. () = number of waivers which were based (also) on results from juvenile animal toxicity studies. [] = number of switches from initial PIP applications to full waivers based on safety concerns (most of these were full waivers on PDCOs own motion). (Source: EMA databases DREAM, PedRA)" [5]. (Source: EMA)

Another impact of JAS findings can be demonstrated by Kalydeco® (ivacaftor) [5]. Kalydeco® has been approved for cystic fibrosis for adults and for children from six years of age. After ophthalmic unobtrusive adult animal studies had been performed, cataracts could be observed in JAS. As a consequence, ophthalmological monitoring was implemented throughout the paediatric clinical trials. Indeed, cataracts could have been identified in children receiving Kalydeco® in clinical trials. Without the appropriate JAS findings, the adverse events might have been missed.

Lastly, recommendations for action could be derived for ophthalmological examinations. The content of the SmPC has been adjusted correspondingly by the inclusion of evaluation criteria and safety concerns for children receiving Kalydeco®.

2.5.2 Value of JAS – The scientific sword of Damocles over regulatory requirements

As evident from the previous section *Clinical and Regulatory Consequences of Definite JAS Findings,* JAS are significant for the overall development of medicines intended for paediatric use. Simultaneously, the value of JAS as a reliable approach for the prediction of toxicities that occur exclusively in the human developing organism, i.e. children, has been discussed extremely controversial. This discussion has been going on since the implementation of JAS in regulatory frameworks.

The EMA justifies JAS as follows: "The main aim of non-clinical studies to support the development of medicinal products to be used in pediatric patients is to obtain information on the potentially different safety profiles from those seen in adults. Juvenile animal studies can be used to investigate findings that cannot be adequately, ethically, and safely assessed in pediatric clinical trials" [9].

However, the majority of demanded JAS corresponding to paediatric age ranges which are not paediatric at all (provided the term paediatric means physically not

mature). More precisely, 57% (24/ 42) of all partial waivers based on safety reasons and arisen in JAS could be assigned to clinical trials intended for children above the age of 12. A closer look at Figure 3 demonstrates impressively that children at the age above 12 are nearly physically mature. This applies particularly to organs/ systems which affect the ADME (e.g., gastrointestinal, and renal system). From 12 years of age, solely the endocrine, nervous, reproductive and skeletal system could be considered as significantly immature. Consequently, most partial waivers based on safety reasons also arisen in JAS were for presumable physically mature human beings (note: the presumption based on Figure 3 requires a final assessment which include, inter alia, the development status of the target organ during the intended drug administration and further factors listed in Section 2.2.1). The first weeks after birth are the really challenging period in human development, especially in preterm newborns. Klaus Rose points out that "Children do not remain as immature and vulnerable as preterm newborns or term newborns until they become administratively adult" [65].

All in all, it is difficult to see how any non-human being (e.g., the rat) could add evidential weight to what is likely to happen in humans? Furthermore, Georg Schmitt writes: "Juvenile animal models are not only inflicted with the common difficulties of species to species translation but also with additional ambiguities to translate postnatal development across species" [66].

A review comprises 241 JAS for small molecules in various species, suggesting that less than 25 % of the studies contributed new data. Furthermore, the authors point out that "Although this may imply that these studies were therefore justified and had an impact on the safety assessment this should be viewed with caution as the simple collection of new data does not necessarily correspond to a better safety assessment unless the data have a clinical relevance" [67]. Even though, JAS findings presumably clinically relevant are included in the drug product labeling "it is unclear how a health care professional would use the presented study findings (often in

technical jargon) when considering prescribing the drug to a child" and "what the differences actually mean when compared with adult animal results" [68].

Several publications call into question the utility of JAS, one of them considers PIP decisions to 229 drugs [41; 65; 68; 69]. Overall, it can be concluded that the medical and scientific sense of JAS has not been proven.

As an example, both false positive and negative JAS findings can be listed. Toxicities are alarming which have occurred in clinical trials after experimental animal studies had identified the respective drugs as "safe". The most prominent example is thalidomide which caused devastating phocomelia in up to 30,000 infants before it was withdrawn. The significant teratogenicity of thalidomide could not be revealed in extensive animal tests (i.e., 10 strains of rats; 11 breeds of rabbit; 2 breeds of dog; 3 strains of hamsters; 8 species of primates; and various cats, armadillos, guinea pigs, swine, and ferrets) [70].

In contrast, animal studies can erroneously declare a drug as unsafe and unjustifiable for further investigation in human trials. The actual number of false positive JAS blocking the development of beneficial medicine remains hidden. Examples are restricted to medicine which had been launched before animal testings were implemented as standard approach within the entire drug development process. Penicillin, paracetamol, and, not least, aspirin for reasons of animal embryo toxicity by far cannot meet current non-clinical requirements and would never have been launched [6; 71].

This section closes with Figure 11 [6]. The figure demonstrated the predictivity of experimental animal studies to human toxicity: "About 12% of pharmaceuticals pass preclinical testing to enter clinical trials. Of those, only 60% successfully complete phase 1 trials. Overall, approximately 89 % of novel drugs fail human clinical trials with approximately one-half of those failures due to unanticipated human toxicity". Van Norman finally asks: "If animal testing accurately predict human toxicity, then why are toxicity-related failure rates in human clinical trials so high?" [6].

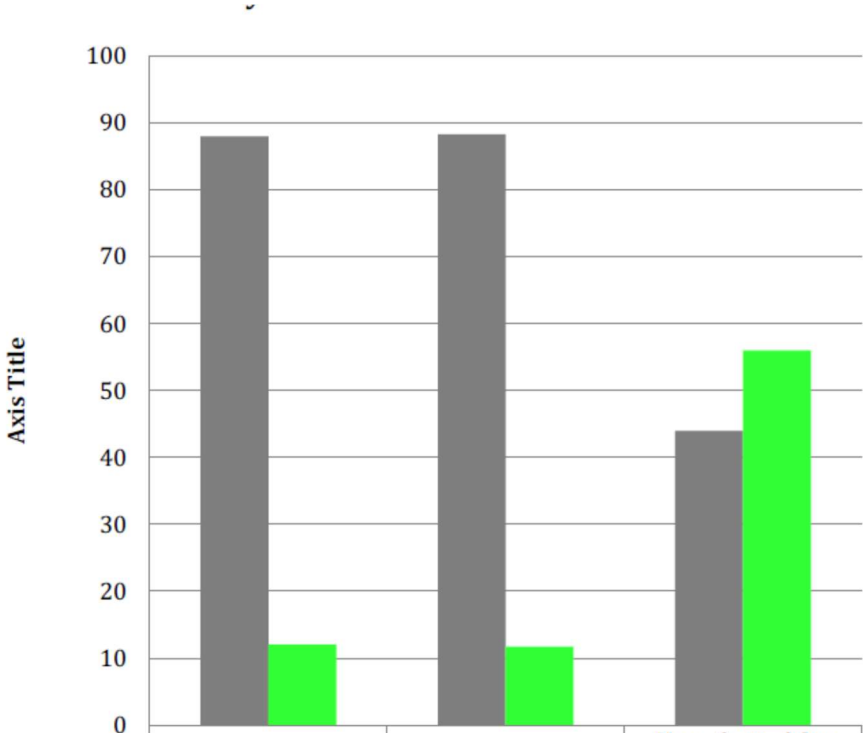


Figure 11. Failure rate in translational research: preclinical and clinical trials. "Percentages of drugs that fail in preclinical trials (due to drug toxicity or failure of efficacy in animal testing [not particular to JAS with its with additional ambiguities to translate postnatal development across species, R. S.] and in clinical trials (due drug toxicity or failure of efficacy in human testing) are shown in column 1 and 2. The third column demonstrates what would happen if animal and human toxicity were closely correlated and therefore drugs with human toxicity were eliminated at the preclinical testing stage by animal toxicity testing (one-half of all drug failures in clinical trials are due to toxicity issues despite safety in animals). Success rates of clinical trials increase from 11.7% overall to approximately 56%" [6]. (Source: *Gail A. van Norman*)

2.6 Timing of JAS in Relation to Clinical Trials – Time is Health

(see Flowchart (6f))

After the WoE outcome justifies a JAS (see Section 2.2), the timing of such studies in relation to clinical trials should be determined (see Figure 12). For this purpose, the ICH M3 (R2) guideline essentially provides two recommendations: JAS should be contemplated "before initiation of short-duration multiple-dose efficacy and safety trials" and "for long-term clinical trials in pediatric populations when an assessment of juvenile animal toxicity is recommended, the nonclinical studies should be completed before the initiation of the trials" [11]. The ICH S11 complements ICH M3' guidance as follows: "For severely debilitating or life-threatening diseases, or diseases with serious unmet medical need in a paediatric population, the sponsor and regulatory agencies should consider the benefit of producing data in addition to existing studies versus the potential delay in patient access to a pharmaceutical caused by additional nonclinical testing," and ICH S11 states further, "The decision whether to perform nonclinical testing and its timing should be based upon a thorough risk-benefit evaluation" [7].

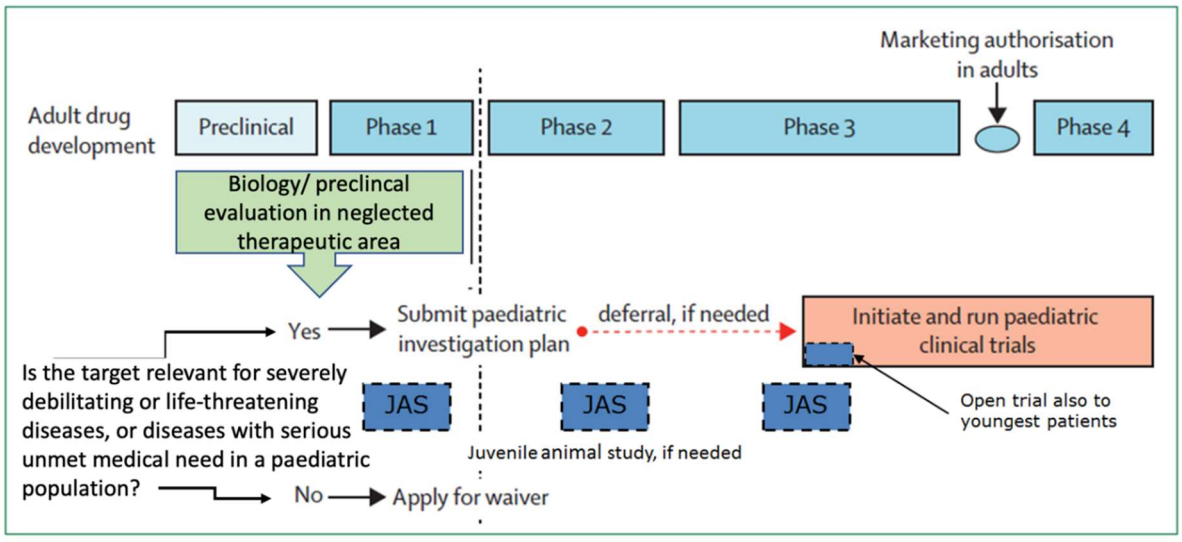


Figure 12: Options for timing of JAS. The timing of JAS (dark blue dotted box) is depicted in relation to the adult drug development (top row). Depending on the degree of unmet medical need (i.e., medical urgency and importance), JAS can be initiated between the paediatric investigation plan submission and with the start of paediatric clinical trials. A

temporal shift of JAS is justified when the unmet medical need outweighing identified safety concerns [43; 72]. (Based on *Vassal et al.* and EMA)

Although the ICH S11 provides further guidance regarding the timing of JAS the following aspects remain uncertain:

1. For whom (i.e., patients) exactly is a temporal shift of JAS warranted?
2. How should a risk-benefit evaluation be applied?

With regard to the first question: According to ICH S11, a temporal shift of JAS is warranted for medicines intended for children also with "serious unmet medical need" [7]. However, when exactly a medical need can be categorised as serious and unmeet is not defined. Therefore, the term unmet medical need can be interpreted differently by different stakeholders. Patients of particular concern are those waiting hopefully for a (better) treatment, demanding fast solutions for demonstrable reasons.

Long before the inception of ICH S11 the expression "unmet medical need" has become a substantial part of several decision-making processes within the regulatory landscape. For instance, the interpretation of the term "unmet medical need" determines whether a conditional marketing authorisation/ accelerated assessment is justified. To that end, Article 4 paragraph 2 of Commission regulation (EC) No. 507/2006 specifies that "'unmet medical needs' means a condition for which there exists no satisfactory method of diagnosis, prevention or treatment authorised in the Community or, even if such a method exists, in relation to which the medicinal product concerned will be of major therapeutic advantage to those affected" [1].

Furthermore, the 'unmet medical need' is one basis for granting an orphan drug designation [73], and waivers in the course of PIP evaluation, because a PIP condition can be waived if "the specific medicinal product does not represent a significant therapeutic benefit over existing treatments for paediatric patients" [1].

However, despite of the importance of the "unmet medical need" for the regulatory decision process in general and more specifically regarding the ICH S11, a tool/ definition to quantify the degree of medical urgency and importance (i.e., unmet medical degree) does not exist. One approach to overcome this challenge could be an economic tool used during the health technology assessment (HTA). The so called quality adjusted life years (QALYs) serves normally as a basis to evaluate whether (or not) a medicine offers good value for money (also in relation to the standard treatment, if available) [74]. The QALYs are consulted for reimbursement negotiations between different stakeholders (e.g., payers). After the marketing authorisation holder (MAH) has declared his intention to launch the medicinal product, the HTA will take place based on clinical trial result.

QALYs takes into account how a treatment affects patients concerned in terms of their quantity (how long you live for) and quality (the quality of your remaining years of life) of life. However, QALYs could be also used to quantify the health status of patients independently of any treatment option. Consequently, QALYs are useful for the quantification of unmet medical needs. How to work out QALYs can be seen on pages provided by NHS Scotland [75].

With regard to the second question: The ICH S11 does not provide any guidance for its demanded risk-benefit evaluation to determine an acceptable timing of JAS. The following new concept could be a basis to overcome the challenge to determine and standardise the timing of JAS (see Figure 13):

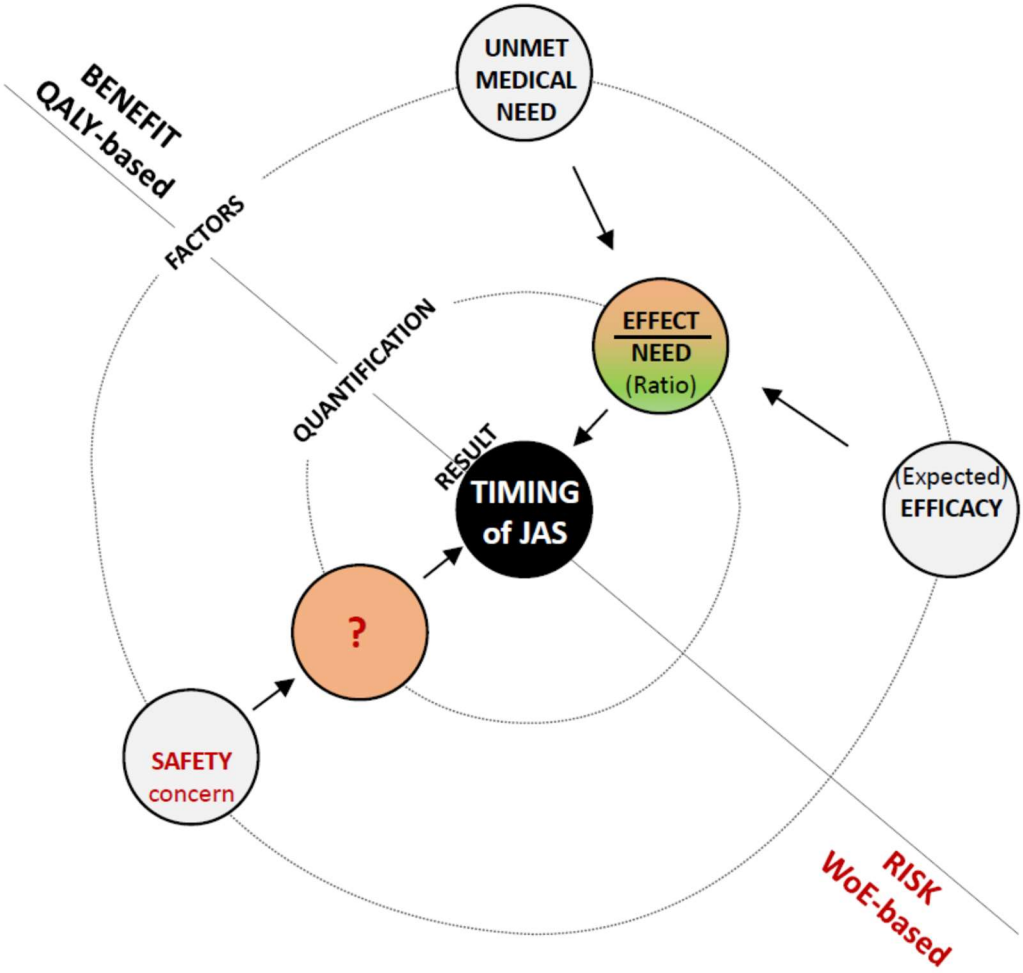


Figure 13: New concept of risk-benefit evaluation to determine the timing of JAS. Firstly, the risk-benefit evaluation is divided into a benefit (upper right half) and a risk (lower left half) assessment towards timing of JAS (result). Regarding the benefit assessment the factors (outer circle) unmet medical need and (expected) efficacy should be consulted. The quantification (inner circle) of benefit factors is QALY-based. The QALY-based ratio of unmet medical need and efficacy indicates the potential value of the investigational drug for the intended paediatric population (this approach required a determination of categorising thresholds). Generally, the medicinal product must address the unmet medical needs to a significant extent and should represent a significant therapeutic benefit over existing treatments for paediatric patients (otherwise, a waiver could be justified). At best, this part of benefit evaluation can be performed based on existing adult data or data to paediatric subsets of higher age. Only if the intended treatment is significantly beneficial for patients concerned a balancing against the safety concerns is necessary. Otherwise, a delay of the clinical trial in paediatric patients can be accepted (i.e., the JAS can be performed as usually prior to paediatric clincal trials). The safety is based on the WoE-outcome, which should be performed anyway. For this purpose, the degree of identified safety concern can be set into relation to a numerical rating scale (a creation of such a scale is not part of this thesis). Finally, an index number resulting from the ratio of benefit (QALY-based ratio of Effect/Need) and safety (WoE-based) indicates the acceptable timing of JAS.

Even though the main focal point of our efforts should be the treatment of human beings and not the treatment of numeric value, the concept described above could be a basis for further discussions and improvements to overcome the highly ethical challenge regarding the timing of JAS. In that discussion, the introduction of health thresholds and categories as well as the determination of the expected efficacy could be seen as the most challenging part. Furthermore, the value of JAS for children concerned should be considered honestly, particularly in the context of circumstances which do not allow any unnecessary delay.

Note: The EMA provides an inventory of the needs for paediatric medicines with the aim to help drug-developers to identify opportunities [59].

2.7 The ICH S11 Driven Paediatric-First/Only Development – A Clear Regulatory Path, Finally

ICH S11' section *considerations for paediatric-first/only development* provides guidance to the nonclinical safety testing of medicines initially developed for paediatric use [6]. The paediatric-first and the paediatric-only development have a lack of data from adult patients in common, as a development in the paediatric population has priority (first) or can only be performed exclusively in paediatric ages (only) (e.g., Neuronal ceroid lipofuscinosis) [76]. The lack of clinical data (adult and/or paediatric) is generally challenging because they can be seen as the most valuable source for the identification of potential safety concerns. Therefore, both approaches (only and first) involve a First-In-Human (FIH) clinical trial with healthy adult volunteers to assess safety and tolerability (see Table 7; general approach/ paediatric-only development). A deviation from the general approach is justified when the disease that only exists in children is, for instance, either debilitating or

life-threatening or the pharmaceutical cannot be administered safely to adult volunteers. In this case, the FIH trial should be performed directly in paediatric populations instead in adults (see Table 7, emergency approach/ paediatric-first development). In any case, supportive Repeated-Dose-Toxicity (RDT) studies must be implemented. The setup of RDT studies needs to be adapted to warrant an appropriate characterisation of "the toxicological profile of the test compound following a repeated administration. This includes an identification of potential target organs of toxicity and exposure/response relationships and may include the potential reversibility of toxic effects" [34]. Primarily, to ensure this ICH S11 recommends the use of two customised RDT studies with varying species (nonrodent and rodent). The setup of such studies is also determined by the used FIH approach: to support paediatric FIH trials (Emergency approach) RDT studies with juvenile animals are strongly recommended; for the general approach adult animals are generally sufficient. In both cases, the pharmacology safety package as well as the assessment of the genetic toxicology could be performed with adult species.

Furthermore, ICH S11 delivers' guidance to special requirements on nonclinical safety studies in context of chronic diseases, biopharmaceuticals and non-human primates, particularly in association with juvenile animals. These recommendations are supposed to complement the both approaches (general and emergency) on a case-by-case basis.

In case of chronic diseases, chronic toxicity studies should be performed to simulate the clinical administration properly. Therefore, according to ICH S11 extended RDT studies in two species (nonrodent and rodent) are needed, whereby "in at least one of these studies, dosing should start at an age developmentally matched to the lowest age of the intended patient population" [7]. Hereinafter, ICH S11 suggests that "In principle, chronic studies that start dosing from ages that developmentally correlate to the youngest paediatric patient age can be sufficient to cover all ages

and durations of paediatric use" [7]. Beside the pharmacology safety and genetic toxicology package the assessment of reproductive toxicity and carcinogenicity can be required [7].

In case of biopharmaceuticals that could be tested in JAS, principles of ICH S6 can apply [77]. That also applies to the assessment of the genotoxic and carcinogenic potential. Moreover, ICH S11 recommends the inclusion of non-invasive safety pharmacology endpoints in juvenile or standard non-human primate (NHP) RDT studies.

In case of NHP for JAS, ICH S11 points out the limitations regarding the use of postweaning pups for JAS. The coverage of the lowest paediatric age ranges is limited, as juvenile NHP are weaned relatively late (at the age of 10-12 month). The use of preweaning NHP should stay an exception; for instance, "in the situation of pharmaceuticals with first and primarily neonatal clinical use, and where alternative approaches to nonclinical safety assessment are not feasible" [7].

Alternatively approaches should only be considered, where a JAS is not feasible to support the youngest paediatric age [7].

Thus far ICH S11, before ICH S11' implementation a series of ICH guidelines have been in force that support the nonclinical development of adult and paediatric medicines [18]. Of great importance is ICH M3 (R2), as it defines the essential nonclinical studies to support the inclusion of healthy adult volunteers before initiating any trials in paediatric patients (FIH) [11]. However, none of these guidelines offer a clear regulatory path for the paediatric development when there are no prior data in adults. Also, high-profile national guidelines primarily deal with the traditional approach, adult first [9; 12; 13]. In many cases, followed by using deferral options to gradually move from adults to the youngest relevant paediatric population.

ICH S11 recommendation regarding the number of species for a warranted JAS is in accordance with already existing paediatric safety guidelines a single species is

usually considered sufficient [7; 9; 12; 13]. An analysis of issued opinions by EMA' PDCO apparently confirms an implementation of these recommendations into practice: the majority (80%) of PIPs with juvenile animal studies (232/881) contained a JAS with only one species [5]. Consequently, about 20% of these PIPs consist of JAS with 2 or more species. According to ICH S11 a paediatric first development (directly targeting paediatric populations without available data in adults) generally requires JAS in two species (see Table 7). Based on preceding data and further analysis to EMAs PIP opinions no reliable statement can be made whether ICH S11 recommendations are consistent with the current practice or not [5; 78; 79]. A compilation of the kind of paediatric development programme (following the adult development, paediatric first or only) and the respective species used for the safety assessment would be helpful. In principle, "a publication of the rationale with details of why juvenile animal work is being proposed by a drug company or requested by the regulators" [79] could ideally reduce the number of JAS and accelerate the overall paediatric drug development. This approach is only possible when beside the publications of data and its rationale ICH S11 suggestions are not considered as irrefutable rule. Unfortunately, in ICH S11' section *considerations for paediatric-first/only development* makes no reference to its WoE review (see Section 2.2). A WoE review should be performed in any case, even though the development of paediatric-only indications, in the absence of any adult data, generally requires two JAS from the outset. The WoE review makes sense, as an "in-depth knowledge of the pharmacology, pharmacokinetics and metabolism, and the disease may negate the need for juvenile animals in the repeat-dose studies" [80]. *Schmitt et al.* point out "that the juvenile rodent study starting dosing from ages that developmentally correlate to the youngest paediatric ages and until sexual maturity could present the only and definitive juvenile study" [80]. Provided this screening design (similar to that used in adult toxicology studies) is technically feasible and does not reveal any safety findings, it could justify the use of only one JAS for the development of paediatric only indications. On the other hand, in the next step (not in parallel) a

second JAS could be performed with a targeted design that addresses emerged safety concerns. This approach is in accordance with ICH S11 recommendations; however, they are restricted to the safety assessment of drugs intended for chronic diseases (see Table 7) [7].

In general, due to the confidential nature of competitors findings in the absence of any clinical data at the time of the first ever dose in paediatric patients a timely exchange with competent authorities (e.g., in the form of a scientific advice) is more than advisable. Not least because of gaining sufficient knowledge to inform the WoE-Review and thus to accelerate the availability of preferably safe and efficient medicine for children.

In conclusion, with its section *considerations for paediatric-first/only development* the ICH S11 sets new regulatory standards. Based on available PIP analysis no statement can be made whether these standards are in line with the general practice. Unfortunately, the universally useful WoE-tool to assess the need and, in last consequence, the kind of additional nonclinical testing are not mentioned. The usage of the WoE review could reduce under certain circumstances the number of JAS, also within the development of paediatric specific indications.

Paediatric First and Only Development (ICH S11 recommendations) [7]	
General approach	Emergency approach
FIH trial (Only): Adult volunteers	FIH trial (First): Paediatric patients
RDT (sub-chronic) study: 2 adult species (rodent and non-rodent); or one or both could be replaced by juvenile species (continued dosing up to maturity) incl. additional endpoints; Advantage: more efficient, could accelerate pivotal studies. Principles of ICH S6 can also apply (s. below) [77].	RDT (sub-chronic) study: 2 juvenile species (rodent and non-rodent); onset of dosing at ages that developmentally correlate to the age of the paediatric patients. Principles of ICH S6 can also apply (s. below) [77].
Further studies: Safety pharmacology and genetic toxicology (in adult animals)	Further studies: Safety pharmacology and genetic toxicology (in adult animals)

<table>
<tr><td colspan="2">Chronic (disease) toxicity studies</td></tr>
<tr><td colspan="2">RDT (chronic) study: 2 species (rodent and non-rodent); at least one juvenile animal study (JAS) where dosing start at an age developmentally matched to lowest age of intended patient population; this JAS could be sufficient when it covers all ages and durations of paediatric use.</td></tr>
<tr><td colspan="2">Further studies: safety pharmacology and genetic toxicology (obligative) as well as assessment of reproductive toxicity and carcinogenic potential can be warranted.</td></tr>
<tr><td colspan="2">Biopharmaceuticals (ICH S6), assessment in JAS</td></tr>
<tr><td colspan="2">RDT study: 2 species (rodent and non-rodent); generally, the test compound should be potentially pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies). When the biological activity is well understood, only one relevant species exists or short-term toxicity studies show similar effects in both species, a longer-term study with one animal could be sufficient. When no relevant species exists, the use of relevant transgenic animals expressing the human receptor, or the use of homologous proteins should be considered [77].</td></tr>
<tr><td colspan="2">Further studies: Genotoxicity and long-term carcinogenicity studies usually are not appropriate [77]. Non-invasive safety pharmacology endpoints can be included in the juvenile or standard NHP repeated-dose studies [7].</td></tr>
<tr><td colspan="2">Non-human primate (NHP) for JAS</td></tr>
<tr><td>Postweaning NHP</td><td>Preweaning NHP</td></tr>
<tr><td>RDT (sub-chronic) study: coverage of the lowest paediatirc age ranges is limited, as weaning age is 10 to 12 months of age.</td><td>RDT (sub-chronic) study: only recommended in the situation of pharmaceuticals with first and primarily neonatal clinical use, and where alternative approaches to nonclinical safety assessment are not feasible.</td></tr>
<tr><td colspan="2">`In cases where a JAS is not feasible to support the youngest paediatric age, alternative approaches (e.g., in vitro assays, genetically-modified animal models, surrogate molecules) should be considered if available and relevant [7].</td></tr>
</table>

Table 7: Paediatric-first/only development according to ICH S11 recommendations. Two approaches can apply: general (paediatric-only development) or emergency (paediatric-first development e.g., for life-threatening or debilitating paediatric disease or where pharmaceutical cannot be given safely to adult volunteers). Essentially, both approaches differ in the First-In-Human (FIH) trial and the supportive Repeated-Dose-Toxicity (RDT) study. These approaches are complemented by recommendations which requires special considerations (i.e., chronic diseases, biopharmaceuticals in JAS and Non-Human-Primate (NHP) for JAS). Principles of ICH S6 can also apply [7; 77].

3 CONCLUSION AND OUTLOOK

To sum up, the *conclusion and* the *outlook* are shown in tabular form:

Conclusion	**Issue**	**Outlook** (Suggestion)
1. The Scope of ICH S11 (see Section 2.1)		
➢ The ICH S11 covers the nonclinical paediatric gap within the ICH-landscape including the regulatory neglected paediatric-first/only development. ➢ The ICH S11 does not apply to all pharmaceuticals (but "some of the thinking […] can apply"). ➢ The terminology used does not always allow a clear assignment of one pharmaceutical to one ICH-guideline.	➢ The ICH S11 is not fully applicable to anticancer drugs (WoE-based justification no, ICH S11 based study design yes) ➢ For vaccines etc., some of the thinking outlined in ICH S11 can be applied (Why? And, what of the thinking?).	➢ The ICH S11 should be fully extended to all drugs to determine if additional safety studies are warranted (or not). Reason: The WoE review is applicable to all kinds of pharmaceuticals. ➢ Regarding to vaccines etc. the ICH S11 should provide a ratio for its "some of the thinking" statement. Only in this way the user can derive an action.
2a The Weight of Evidence (WoE) Review (see Section 2.2)		
➢ The WoE review is an innovative decision-making tool to evaluate whether additional nonclinical safety studies are warranted. It consists of quality (factors) and quantity (case examples) criteria that should ensure a standardised approach. ➢ Part of the WoE review is high-level overview to the age-depended organ development of humans that assists in the identification of safety concerns.	➢ According to the WoE figure/ tool, the WoE factors *Youngest Intended Patient Age* and *Clinical Treatment Duration* have to be weighted gradually. The gradual weighting conflicts with scientific findings (e.g., CNS-system of adolescent is potentially increased vulnerable) and provided information (i.e., short time exposure can have deleterious effects) and hampers a sufficient single case consideration.	➢ The first factor should be replaced by a factor called *organs/systems of relevance development during/after treatment*. This should sharpen the focus towards the organ development of relevance, as its vulnerability does not necessarily correlate gradually with the patients' age. ➢ To involve harmful effects as a result of a short time exposure the latter factor should at least be reconsidered. ➢ Overall, an adjustment of both factors should enable a sufficient single case assessment (away from a non-sufficient probably based estimation)
2b WoE outcome (see Section 2.2.3)		
➢ ICH S11 insists on the consideration of "the totality of the evidence", i.e., the implementation of the complex WoE review. At the	➢ The conduction of the WoE review can be complex and therefore prone to errors (e.g., different users can come to different results).	➢ A compilation of single factors of sufficient magnitude and clear and common cases would help to simplify further and

same time, ICH S11 provides a set of relatively simple single cases where additional nonclinical safety studies are warranted (or not) ➢ The objectives of the study are required to be aligned with both the WoE outcome and the intended paediatric use.		standardise the decision-making process. This compilation should be placed in front of WoE-review. In case of further uncertainties, the WoE-tool could be used to ascertain whether the evidence warrants further nonclinical investigations or not.
3. Additional Nonclinical Safety Studies (see Section 2.3)		
➢ ICH S11 essentially provides only to JAS further guidance (choice of species, design, treatment initiation etc.) ➢ The medical and scientific sense of JAS has not been finally proven (see Section 2.5) ➢ On EU-level, several efforts have been made to strengthen the development of alternative approaches to JAS such as the possibility to submiss data to alternative testing approaches on a voluntary basis beside to data obtained by regulatory accepted methods. ➢ To date, no alternative testing approaches have been regulatory accepted which could replace JAS. ➢ Besides its limitations (e.g., NOAEL), the generally regulatory accepted PBPK modelling is not yet sufficiently accurate for the paediatric, particularly neonate drug development. ➢ The MPS technology is under development, but in the long-term it can surpass by far JAS regarding its predictivity und required efforts (in terms of cost and time) ➢ According to ICH S11, mPPND should be considered (if possible). However, it does not provide further guidance to usual mPPND challenges.	➢ The value of JAS is obviously limited (see Section 2.5) and binds lots of resources (cost and time) which could be used to cover important concerns such as the neglected therapeutic needs in the flied of paediatrics. ➢ The guidance to mPPND studies does not go beyond existing recommendations. It remains uncertain how to deal with challenges such as the direct exposure of juvenile animals to the drug in another way than via maternal milk.	➢ Due to the limited predictivity of JAS regarding the paediatric population, more effective measures should be undertaken to replace JAS as default approach. These measures could include an obliged submission of data obtained by an alternative testing method to JAS beside the already regulatory accepted data. This approach could be restricted to companies with appropriate infrastructure and the high savings potential. A nonclinical approach with high predictivity for the paediatric population as presumably the MPS technology under development could reduce the cost and time per drug development programme significantly; these could encourage drug developers to invest in projects which would cover neglected therapeutic needs with a small target group. ➢ Regarding mPPND, further guidance to common mPPND challenges should be implemented in the ICH S11.

4. Juvenile Dose Ranging Approach (see Section 2.4)		
➢ A dose range finding (DRF) study in juvenile animals is usually recommended to increase the quality of the definitive JAS. ➢ Dosages used in the adult toxicology studies are often not informative regarding the tolerated dose levels in juvenile animals. ➢ The ICH S11 provides a detailed explanation to DRF studies in general.	➢ JAS are relatively often requested in support of trials with children below the age of two, particularly to the age range corresponding to the preweaning period. However, the ICH S11 do not provide a clear dose range finding approach for the challening studies with preweaned animals.	➢ Because of the particular importance for the qualitiy of definitive JAS and finally the interpretation of data for the corresponding paediatric population, an implementation of the phased DRF approach with preweaned animals into the ICH S11 should at least be considered.
5. Consequences and Value of JAS (see Section 2.5)		
➢ Findings of JAS can have direct implications on PIPs (e.g., granting of full or partial waivers), the safety monitoring (e.g., adjustments) and SmPCs and/or EPARs (e.g., inclusion of reports/warnings). ➢ It is unclear how a health care professional would interpret safety findings in SmPCs when considering prescribing the drug to a child. ➢ In contrast to the claim that animal testing accurately predicts human toxicity, the toxicity-related failure rates in human clinical trials are still high. ➢ The medical and scientific sense of JAS has not been finally proven.	➢ The value of JAS findings, and thus also the derived consequences are still questionable	➢ See point 3 of this section
6. Timing Of JAS (see Section 2.6)		
➢ ICH S11 states, the timing of the additional nonclinical safety studies should be based upon a thorough risk-benefit evaluation. ➢ Particularly, "for severely debilitating or life-threatening diseases, or diseases with serious unmet medical need in a paediatric population, the sponsor and regulatory agencies should consider the	➢ The ICH S11 do not provide any guidance for its demanded risk-benefit evaluation to determine an acceptable timing of JAS. ➢ Terms such as serious, particularly in the context of the unmet medical need (UMN) are not sufficiently defined for a quantification of the UMN. This hampers the	➢ To overcome this challenge a new concept has been presented that could serve as a basis for further discussion and improvements. The new concept to determine an acceptable timing of JAS comprises a WoE (safety) and QALY (benefit) based assessment.

benefit of producing data in addition to existing studies versus the potential delay in patient access to a pharmaceutical caused by additional nonclinical testing" (ICH S11).	necessary determination of an acceptable timing of JAS.	
7. Paediatric First/Only Development (see Section 2.7)		
➢ The paediatric-first and the paediatric-only development have a lack of data from adult patients in common, as a development in the paediatric population has priority (first) or can only be performed exclusively in paediatric ages (only). ➢ So far, this part has been neglected by regional guidelines. However, the ICH S11 sets with its section *considerations for pediatric-first/only development* new regulatory standards. ➢ Both approaches (first and only) generally comprise two animal studies. These should be JAS in the paediatric-first development as clinical trial starts with children.	➢ Under certain circumstances one JAS can be sufficient to address concerns with regard to the paediatric-first/ only development (e.g., when dosing starts from ages that developmentally correlate to the youngest paediatric age and continues until sexual maturity). ➢ The ICH does not suggest the implementation of the WoE review in the context of the paediatric-first/ only development to assess the need of two animal studies.	➢ The WoE review should also be performed in the context of the paediatric-first/ only development to assess the necessity of a second animal study after the first one (screening design) has been completed.

Table 8: Conclusion and outlook.

4 SUMMARY

With the introduction of paediatric specific legislation, the paediatric drug development has become a compulsory part of the adult drug marketing application in North America and Europe. Since then, the nonclinical drug development programmes tailored to children have gained in importance. The conduct of additional nonclinical toxicity studies can be justified to get knowledge about potentially different safety profiles from those seen in adults. However, the appropriate EU, US guidance and even ICH M3 do not provide clear guidance to the need and design of such studies. These lack of further guidance and harmonisation facilitated the conduct of similar animal studies between regulatory regions without substantial added value and hampered a preferably quick and wide availability of medicines for children. To overcome these challenges the ICH issued the new safety guideline *Nonclinical Safety Testing in Support of Development of Paediatric Pharamceuticals,* also called ICH S11.

The aim of the ICH S11 is to set "international standards for, and promote harmonisation of, the nonclinical safety assessments to support the development of pharmaceuticals intended for paediatric use" (ICH S11).

The aim of this master thesis is a critical review of ICH S11' guidance on nonclinical safety testing and their consequences for the overall development of paediatric medicines.

For this purpose, the scope of the guidance, the new decision-making tool for determining the need of additional nonclinical safety studies (i.e., Weight of Evidence (WoE) review) was considered as well as alternatives to juvenile animal studies (JAS), the approach of dose range finding studies, and the consequences, value and timing of JAS and the recommendations to the paediatric-first/only development.

Taken together, the ICH S11 surpasses by far already existing paediatric nonclinical safety guidelines regarding the extent and depth of provided information. Above all, this includes its appendices, i.e., a high-level *overview of age dependent development of organ systems by species* and table a to *principal advantages and disadvantages of various mammalian species for use in JAS*. This information should assist in the identification of potential safety concerns, the choice of species and the timing of JAS to corresponding clinical treatment. Particularly noteworthy is ICH S11' newly decision-making tool, i.e., the WoE review. The WoE review should be performed to determine whether additional nonclinical safety studies are warranted (or not). Beside clear decision criteria, the ICH S11 provides four single cases to ensure the most standardised approach possible. The study objectives should be aligned with the WoE outcome and the intended paediatric use. Furthermore, unlike to the appropriate US and EU guidance the ICH S11 set standards to medicines initially developed for paediatric use (i.e., paediatric-only/first development).

However, the discussion of the results has led to the following suggestions:

1. The extension of the WoE review to all kinds of pharmaceuticals (as scientifically applicable for all pharmaceuticals) and the paediatric-first/only development (as under certain circumstances fewer animal studies than recommended can be warranted).
2. The adjustment of two WoE factors to sharpen a single case consideration (away from probable estimation).
3. Generally, the implementation of more effective measures which could result in regulatory accepted alternative approaches to JAS (which could, in turn, lead to neglected therapeutic needs in more drug development programmes).
4. A new concept to determine an acceptable timing of JAS (how to determine an acceptable timing remains unknown).

5. The implementation of a phased DRF approach with preweaned animals (as JAS to corresponding paediatric population is particularly challenging and relatively often requested (2 ≤ years of age)).

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6 APPENDIX

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Search:

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
	All	All		["D ⊗	All	All	All	All
⊕	Protocol	Embryotoxicity Testing in Post-Implantation Whole Embryo Culture (WEC) - Method of Piersma (.\DBALM_docs \123_P_Embryotoxicity Testing in Post-Implantation Whole Embryo Culture.pdf)	123	Developmental toxicity	EMBRYO CULTURE	Cell viability Embryo functionalities Embryo growth Embryo morphology	Whole embryo culture (rat)	
⊕	Protocol	The Micromass Test - Method of Brown (.\DBALM_docs \122_P_The Micromass Test - Method of Brown.pdf)	122	Developmental toxicity	Animal Origin Primary Cell Culture	Cell differentiation Cell proliferation Cell viability Vital dye uptake: Neutral Red (NRU)	Embryonic limb bud cultures (mouse, rat, chick)	
⊕	Method summary	The Micromass Test - Summary (.\DBALM_docs \122_M_The Micromass Test.pdf)	122	Developmental toxicity	Animal Origin	Apoptosis Cartilage maturation Cell adhesion Cell cycle analysis Cell proliferation Cell viability Cell-cell contact: gap junction integrity Cellular functional parameters DNA damage: oxidation Generation of Reactive Oxygen Species (ROS) Incorporation of radiolabels	Embryonic limb bud cultures (mouse, rat, chick) Undifferentiated embryo CNS cells (rat, mouse, chicken) Undifferentiated embryo heart cells (chicken)	
	Protocol	In Vitro Micromass Teratogen Assay (.\DBALM_docs \114_P_In Vitro Micromass Teratogen Assay.pdf)	114	Developmental toxicity	Animal Origin Primary Cell Culture	Cell differentiation Cell number Cell proliferation Cell viability Vital dye uptake: Neutral Red (NRU)	Embryonic limb bud cultures (mouse, rat, chick) Embryonic rat mid- brain (mesencephalon) culture	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
⊕	Method summary	Embryonic Stem Cell Test (EST) - Summary (.\DBALM_docs \113_M_Embryonic Stem Cell Test.pdf)	113	Developmental toxicity	Animal Origin Human Origin Stem Cells	Apoptosis Cell cycle analysis Cell differentiation Cell function: membrane damage Cell proliferation Cell viability Cellular functional parameters DNA damage: strand breakage Gene expression profiling (omics techniques) Gene expression profiling (omics techniques) Generation of Reactive Oxygen Species (ROS) Metabolite profile (omics techniques)	3T3 - Mouse fibroblasts D3 - Mouse embryonic stem cells Embryonic stem cells (ES)	ReProTect
⊕	Protocol	Embryonic Stem Cell Test (EST) (.\DBALM_docs \113_P_Embryonic Stem Cell Test.pdf)	113	Developmental toxicity	Stem Cells	Cell differentiation Cell proliferation Cell viability Vital dye reduction: MTT to formazan	BALB/3T3 clone A31 - Mouse fibroblast cell line D3 - Mouse embryonic stem cells	ReProTect
	Protocol	Rat Whole Embryo Culture (WEC) (.\DBALM_docs \72_P_Rat Whole Embryo Culture (WEC).pdf)	72	Developmental toxicity	Animal Origin EMBRYO CULTURE	Cell differentiation Embryo growth Embryo morphology Embryo viability	Whole embryo culture (rat)	
	Method summary	Post-implantation Whole Embryo Cultures (WEC) - Summary (.\DBALM_docs \68_P_Embryotoxicity testing using a whole embryo culture (WEC) procedure.pdf)	68	Developmental toxicity	EMBRYO CULTURE	Apoptosis Cell cycle analysis Cell viability Cellular functional parameters Embryo development Embryo growth Embryo viability Gene expression profiling (omics techniques) Gene expression: Biomarkers Gene mutation Generation of Reactive Oxygen Species (ROS) Heart functional parameters	Post-implantation whole embryo culture (rat, mouse, hamster, rabbit)	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
	Protocol	Embryotoxicity testing using a whole embryo culture (WEC) procedure (.\DBALM_docs \68_P_Embryotoxicity testing using a whole embryo culture (WEC) procedure.pdf)	68	Developmental toxicity	EMBRYO CULTURE	Cell differentiation Cell morphology Embryo growth Embryo viability	Whole embryo culture (mouse)	
	Protocol	Lung Cell Assay (.\DBALM_docs \48_P_Lung Cell Assay.pdf)	48	Developmental toxicity	Animal Origin Cell Lines Human Origin	DNA synthesis Incorporation of radiolabels Protein synthesis	HFL1 - Human fatal lung fibroblasts cell line L2 - Rat lung ephitelial cell line	
	Protocol	Rabbit articular chondrocyte functional toxicity test (.\DBALM_docs \41_P_Rabbit articular chondrocyte functional toxicity test.pdf)	41	Developmental toxicity	Animal Origin	Cellular functional parameters	Primary culture of articular chondrocytes (rabbit)	
	Method summary	Aggregate culture systems - Summary (.\DBALM_docs \M_Aggregate culture systems.pdf)	0	Developmental toxicity		Cell differentiation Cell function: membrane damage Cell proliferation Protein content: glial fibrillary acidic protein (GFAP) Protein content: myelin basic protein (MBP) Protein content: total	Embryo brain cells (chick, mouse, rat) Embryo neural retina cells (chick)	
	Method summary	Caenorhabditis elegans Model - Summary (.\DBALM_docs \M_Caenorhabditis elegans Model.pdf)	0	Developmental toxicity	Animal Origin	Larval length (*C. elegans*) Reproductive performance	Nematode worm (*Caenorhabditis elegans*)	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
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	Method summary	Chicken Embryotoxicity Test (in ovo and ex ovo) - Summary (.\DBALM_docs \M_Chicken Embryotoxicity Test.pdf)	0	Developmental toxicity	Animal Origin	Apoptosis Cell differentiation Cell migration Cellular functional parameters DNA damage: oxidation Embryo development Embryo growth Embryo morphology Embryo viability Gene expression: Alpha actin Gene expression: Aryl hydrocarbon receptor (AhR) Gene expression: Beta actin Gene expression: Beta-adrenergic receptor Gene expression: Biomarkers Gene expression: Cytochrome P450 isoenzymes (CYP450) Gene expression: Homeobox protein ANF Gene expression: Hypoxia-inducible factor 1 (HIF1A) Gene expression: Insulin-like growth factor-1 (ILGF-1) Gene expression: Uridine glucuronosyltransferase (UGT or UDPGT) Gene expression: Vascular endothelial growth factor (VEGF) Generation of Reactive Oxygen Species (ROS) Heart functional parameters Protein synthesis	Chick embryo in ovo Whole embryo culture (chicken)	
	Method summary	Culture of Palatal Shelves - Summary (.\DBALM_docs \M_Culture of Palatal Shelves.pdf)	0	Developmental toxicity	Animal Origin Cell Lines Human Origin	Apoptosis Cell morphology Cell proliferation Cellular functional parameters Embryo growth	Embryonic palatal shelves culture	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
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⊕	Method summary	Cultures of Embryonal Carcinoma Cells - Summary (.\DBALM_docs \M_Cultures of Embryonal Carcinoma Cells.pdf)	0	Developmental toxicity	Animal Origin Cell Lines	Cell differentiation Cell morphology Cell proliferation Cell viability Cell viability Gene expression profiling (omics techniques) Gene expression: Biomarkers	F9 - Mouse embryonal carcinoma cell line Mouse embryonal carcinoma cells Mouse F9 and P19 embryonal carcinoma cells co-cultured with END-2 cell line P19 - Mouse embryonal carcinoma cell line	
	Method summary	Embryonic Palate Mesenchymal Cells Cultures (primary cells and cell lines) - Summary (.\DBALM_docs \M_Embryonic Palate Mesenchymal Cells.pdf)	0	Developmental toxicity	Animal Origin Cell Lines Human Origin	Apoptosis Cell cycle analysis Cell proliferation Cell viability Generation of Reactive Oxygen Species (ROS)	HEPM - Human embryonal palatal mesenchymal cells Primary murine embryonic maxillary mesenchymal cells Primary murine embryonic palatal mesenchymal cells	
	Method summary	Extended One Generation Reproductive Toxicity Study - Summary (.\DBALM_docs \M_Extended One Generation Reproductive.pdf)	0	Developmental toxicity	IN VIVO MODELS Reduction	Blood: functional and biochemical parameters Body weight Developmental effects Developmental effects Developmental effects Mating performance and pregnancy Oestrus cyclicity Organ weight Organism behavior (clinical observation) Sperm function Tissue histopathology Tissue histopathology Urine content	Rattus norvegicus- Rat	
	Method summary	Fetal Mouse Salivary Glands Cultures - Summary (.\DBALM_docs \M_Fetal Mouse Salivary Glands Cultures.pdf)	0	Developmental toxicity	Animal Origin Salivary Glands	Apoptosis Cell proliferation Lobe formation in fetal mouse salivary glands Morphological effects Protein expression	Salivary glands culture (mouse)	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
	Method summary	Genetically Modified Embryonic Stem Cells (ReProGlo) - Summary (.\DBALM_docs \M_Genetically Modified Embryonic Stem Cells.pdf)	0	Developmental toxicity	Animal Origin	Cell differentiation Cell viability Gene expression profiling (omics techniques) Gene expression profiling (omics techniques)	3T3 - Mouse fibroblasts D3 - Mouse embryonic stem cells Embryonic stem cells (ES)	ReProTect
	Method summary	Hydra attenuata - Summary (.\DBALM_docs \M_Hydra attenuata.pdf)	0	Developmental toxicity		Cnidaria viability Embryo development Embryo viability *Hydra* regeneration	Hydra - adult and isolated regions of the body	
	Method summary	Limb Buds Cultures - Summary (.\DBALM_docs \M_Limb Buds Cultures - Summary.pdf)	0	Developmental toxicity	Animal Origin EMBRYO CULTURE	Apoptosis Cell differentiation Embryo growth Embryonic tissue malformation Gene expression profiling (omics techniques) Gene expression profiling (omics techniques)	Embryonic limb bud cultures (mouse, rat, chick)	
	Method summary	Mouse embryonic tooth explants culture - Summary (.\DBALM_docs \M_Mouse embryonic tooth explants culture.pdf)	0	Developmental toxicity	Animal Origin EMBRYO CULTURE Primary Cell Culture	Apoptosis Cell morphology Cell viability Gene expression profiling (omics techniques) Gene expression profiling (omics techniques)	Embryonic tooth explants cultures	
	EU project	EU Integrated Project - ReProTect - Development of a Novel Approach in Hazard and Risk Assessment for Reproductive Toxicity by a Combination and Application of In Vitro, Tissue and Sensor Technologies. (.\DBALM_docs \PR_reprotect.pdf)	0	Developmental toxicity				ReProTect

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
	Method summary	Placenta Cells and Explants Cultures - Summary (.\DBALM_docs \M_Placenta Cells and Explants Cultures.pdf)	0	Developmental toxicity	Cell Lines Human Origin	Apoptosis Cell differentiation Cell migration Cell viability Cellular functional parameters Gene expression profiling (omics techniques) Generation of Reactive Oxygen Species (ROS)	BeWo - Human choriocarcinoma cell line Cell line cultures Human primary cytotrophoblasts JEG-3 - Transformed human choriocarcinoma cell line Placenta explants Rcho-1 - Rat trophoblast stem cell line SV40-transformed human trophoblast cell lines	ReProTect
	Method summary	Placental Perfusion Systems and Related Test Systems - Summary (.\DBALM_docs \M_Placental Perfusion Systems and Related Test Systems.pdf)	0	Developmental toxicity	Animal Origin Cell Lines Human Origin	Cellular functional parameters DNA damage: adduct formation Enzyme activity: Cytochrome P450 oxidoreductase (CYP) Gene expression profiling (omics techniques) Hormone secretion Metabolic activity Morphological effects Tissue accumulation Transplacental transfer rate Vascular effects	BeWo - Human choriocarcinoma cell line Placenta perfused (human)	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
	Method summary	Pre-implantation embryo cultures - Summary (.\DBALM_docs \M_Pre-implantation embryo cultures.pdf)	0	Developmental toxicity	Animal Origin EMBRYO CULTURE	Apoptosis Cell adhesion Cell function: cytoskeleton Cell number Cell proliferation Cellular functional parameters Chromosomal damage: micronuclei formation Chromosomal damage: micronuclei formation DNA damage: sister chromatid exchange (SCE) DNA damage: strand breakage DNA damage: strand breakage Embryo development Embryo morphology Gene expression profiling (omics techniques) Gene expression: Biomarkers Generation of Reactive Oxygen Species (ROS) Protein synthesis	Pre-implantation whole embryo culture	ReProTect
	Method summary	Sea Urchin Model - Summary (.\DBALM_docs \M_Sea Urchin Model.pdf)	0	Developmental toxicity	Animal Origin EMBRYO CULTURE	Apoptosis Morphological effects	Sea urchin	
	Method summary	The Drosophila Model - Summary (.\DBALM_docs \M_The Drosophila Model.pdf)	0	Developmental toxicity	Animal Origin	Developmental stage: *D. melanogaster* Egg hatching: *D. melanogaster* Enzyme activity Fecundity Gene expression profiling (omics techniques) Generation of Reactive Oxygen Species (ROS) Morphological effects Reproductive performance Survival of adult fly (*D. melanogaster)*	Fuit fly (*Drosophila melanogaster*)	

	Document type	Title	No.	Topic area	Models and Strategies	Biological endpoints	Experimental systems	EUProject
				⊗				
	Method summary	The frog embryo teratogenesis assay: Xenopus (FETAX) - Summary (.\DBALM_docs \M_The frog embryo teratogenesis assay.pdf)	0	Developmental toxicity	EMBRYO CULTURE	Apoptosis Cell function: membrane damage Embryo development Embryo growth Embryo morphology Embryo motility Embryo pigmentation Enzyme activity: AcetylCholinEsterase (AChE) Gene expression: Biomarkers Gene expression: N-cadherin Larvae viability Morphological effects Oocyte fertilisation	Amphibians	
	Method summary	Zebrafish Embryo Assay for Developmental Toxicity - Summary (.\DBALM_docs \M_Zebrafish Embryo Assay for Developmental Toxicity.pdf)	0	Developmental toxicity	EMBRYO CULTURE	Developmental stage: zebrafish Embryo viability Gene expression profiling (omics techniques) Morphological effects	Fish embryo culture (medaka, zebrafish, Oryzias latipes)	

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