16 April 2019
EMA/213997/2019

Overview of comments received on Draft ICH guideline S11 on nonclinical safety testing in support of development of paediatric medicines (EMA/CHMP/ICH/616110/2018)

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

Stakeholder no.	Name of organisation or individual
1	International Council on Animal Protection in Pharmaceutical Programs (ICAPPP)
2	Gilead Sciences International Ltd
3	Swissmedic
4	EFPIA
5	Members of the European Paediatric Translational Research Infrastructure (EPTRI) consortium: Consorzio per Valutazioni Biologiche e Farmacologiche (CVBF) Fyziologicky Ustav Akademie Ved Ceske Republiky Verejna Vyzkumna Instituce (IPHYS)
6	EUCROF

Please note that comments will be sent to the **ICH S11 EWG** for consideration in the context of Step 3 of the ICH process.

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1. General comments – overview

Stakeholder no.	General comment (if any)
1	The ICAPPP appreciates the idea behind this guideline, which aims to provide internationally harmonised guidance on the nonclinical studies recommended to support the development of paediatric medicines. However, considering that one of the stated key objectives of the guideline is to promote a reduction in the use of animals in accordance with the 3Rs principles, we have some serious concerns regarding; 1) the lack of examples and limited guidance provided on nonclinical testing methods other than juvenile animal studies (JAS), and 2) the unsubstantiated support for JAS as a standard approach, rather than as a last resort option. We urge against JAS studies being performed as a 'tick-box' exercise or default option for addressing safety concerns. Considering that the data generated may be of little relevance, the use of the JAS method (especially routinely) could be considered unethical as it may provide false reassurance regarding safety but will certainly cause significant suffering of animals.
	1. Limited guidance on other nonclinical testing methods The title of the guideline is "nonclinical safety testing in support of development of paediatric medicines" and not 'juvenile animal testing in support of paediatric medicines'. Therefore, we would expect to see more guidance on other nonclinical testing methods that should be considered before recommending JAS. Examples of non-animal alternative methods to JAS, which still permit safe and effective drug development and use, should be included in the guideline. Section 2 of the guideline describes the importance of conducting a weight-of-evidence (WoE) analysis as a first step to determine the need for additional nonclinical studies. We support this approach and appreciate the emphasis on the use of existing information from both human and animal studies, pharmacological properties, and data from pharmacokinetic (PK) modelling and computer simulations. If the WoE analysis suggests that additional nonclinical studies are warranted, the guideline states that further in vitro or ex vivo investigations or tests in juvenile animals will be needed. However, the guideline then jumps to a section on the 'design of nonclinical juvenile animal studies' without any further discussion, examples or guidance on the use of the aforementioned in vitro/ex vivo methods. With the goals of minimising animal testing in mind, a section should be added in between Section 2 and Section 3 to provide guidance on the use of in vitro and ex vivo methods to support the development of paediatric medicines. It should also be made clear that, in accordance with the 3R principles, these methods must be prioritised before considering JAS, which have yet to prove their value (see comments below) and should be viewed as a last resort option under very rare circumstances.
	2. Unsubstantiated support for JAS as a standard approach, rather than as a last resort According to the 'background' section, the guideline reflects current thinking based on

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	collations of examples from regulatory agencies, industry surveys and literature. None of these references are provided in the text and we feel that it would be useful to include some of these key sources so that readers can better understand the rationale behind the guideline's recommendations. For decades, the use of medicinal products in children has mostly been determined based on clinical experience with the drug in adults, factoring the dose by a child's body weight (Anderson et al., 2009). However, due to recent changes in regulatory thinking, concerns with off-label drug use and unpredicted toxicities in the paediatric population, and a rise in the number of drug development programs focusing on paediatric-only indications, the number of JAS being requested by regulators and/or conducted by drug developers has increased exponentially over the past 10 years (Downes, 2012). While we appreciate the need to improve the way drugs are regulated and used in the vulnerable paediatric population, there is little evidence to support the use of juvenile animals as a reliable or practical solution. If data from human adults is not enough to predict safety in human children, it is difficult to see how extrapolation of data from young animals to young humans can be meaningful, especially considering the vast species differences (e.g. shorter lifespan, varying developmental schedules etc.) that must be accounted for. "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" (Schmitt, 2015). The difficulty in predicting safety in human children based on data from human adults calls into question extrapolation even between similar groups within the same species; the extrapolation between different species in the JAS method is likely to be even less relevant. We are concerned that JAS are becoming an accepted part of the safety assessment package for new drugs even though their true utility has not yet been fully characterised (Baldrick, 2018). According to the literature, there are not enough clear-cut examples to determine whether JAS are useful or necessary to support paediatric drug development (Baldrick, 2010). Where reviews into the utility of JAS have been conducted, the results are far from satisfactory: Data from 39 JAS, conducted by ten pharmaceutical companies in a variety of species, were compiled and analysed (Bailey & Marien, 2009). Novel toxicity was only observed in four out of the 39 studies compiled, one of which could have been predicted from the pharmacology data. The review found that only in 20% of the studies was it felt that JAS contributed to the paediatric clinical trials and that the JAS were considered to have contributed to the product label in only 30% of cases. "The general perception was that despite these studies, we were not generating anything new; there was no clear indication of novel toxicity or sensitivity; and the findings that were observed were predictable from the known pharmacology, toxicology and the stage of development". The authors concluded that it "could be considered disappointing, in view of the time, number of animals, complexity and cost of the studies, that only between one in three and one in five studies generate data that makes a difference" and that "it would be a terrible waste of time, animals and money, if we perform these

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	studies for no benefit". Another study looked at data from 241 JAS, conducted by 24 pharmaceutical companies, predominantly covering small molecules in a variety of species to support registration of drugs (Bailey & Marien, 2011). The authors found that the JAS contributed to the paediatric clinical trials in 12% and 14%, respectively for the rat and dog and the JAS contributed to the product label in 16% and 19% of cases. In 75% of the rat JAS, all the results were predictable from either the pharmacology (56.9%) or the adult toxicity data (68.1%) and in the dog JAS this was 85.7% (pharmacology – 76.2%, adult toxicity data – 76.2%), which suggests that the studies only contributed new data in less than 25% of cases. "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". The authors concluded that "in view of the huge cost in animals, the financial and time implications, the ethical view (3R) and the complex nature of these studies one could ask if we are doing smart science?". One article stated that "it is currently not clear if there are many (or genuinely any?) clear examples when juvenile animal toxicology studies predicted novel human toxicities that would have an impact in paediatric medicine" and that "animal use (especially in puppies or young monkeys) with no clear goal for risk assessment is totally unacceptable" (Baldrick, 2010). The article also highlighted the need for "push-back" to occur to regulators for requesting JAS if not felt fully justified. Another paper has suggested that the contribution of JAS for "the detection of novel toxicities remains questionable" (Soellner & Olejniczak, 2013). The authors point out that the usefulness of the results from these studies for paediatric development remains unclear and that interpretation of the data and extrapolation to the paediatric population remains difficult. One recent study specifically considered the potential value of JAS in dose selection and safety monitoring of 21 molecularly targeted agents for which human adult data were available (Visalli et al., 2018). Their analysis showed that "JAS are not needed in order to safely conduct Phase 1 trials in paediatric subjects, either for selecting the starting dose or informing on potential toxicities that may be unique to a paediatric population". Importantly, this study concludes that "in the absence of case examples showing that findings of JAS allowed clinical catastrophes to be avoided, we do not believe that JAS provide any value in this setting" and that "abandoning the practice of routinely conducting JAS for most molecularly-targeted oncology drugs would expedite testing in paediatric oncology patients and allow precious drug development resources utilised for JAS to be applied to other promising agents". This makes the vital point that time and money are being wasted on ineffective JAS that could be better used in more effective testing methods which could accelerate drug development. According to a review of EU Paediatric Investigation Plan (PIP) decisions covering the period of 2007 to 2013, it was not clear how many JAS are "actually needed or indeed how useful they are as a means of allowing safe administration of the drug in a

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	paediatric population" (Baldrick, 2018). The author also pointed out that despite increasingly being included in drug product labels, "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". The review concluded that JAS should be strictly avoided as a default, for box-ticking reasons or even to give "comfort factors" for safe use. There are also those that believe that the traditional approach is sufficient; "from decades of clinical pharmacology research of the use of marketed drugs in children we know about the differences of absorption, distribution, metabolism and excretion in the maturing body of the child" (Rose, 2011) and that "despite the lack of paediatric studies, there are many drugs that have been used safely in children" (Anderson et al., 2009). As well as the many scientific issues outlined in numerous review studies such as these, JAS pose a significant animal welfare burden due to the use of vulnerable young animals and the length of time for which the animals are in the laboratory environment. While the severity of these studies is somewhat acknowledged in the 'notes' section of the guideline (e.g. "the propensity for mortality to occur is generally higher in juvenile animals compared to adult animals"), we feel it should be emphasised within the main text of the guideline and that the use of JAS, especially multiple studies in one or more species, should be discouraged (see specific comments on text below). Based on the available evidence, it is difficult to understand why regulators seem to be encouraging the use of JAS and why this draft guideline places so much emphasis on the design of a study that runs counter to the 3Rs. Instead of promoting unreliable and inhumane science, this guideline should be used as an opportunity to steer regulators and drug developers in the right direction and deter unnecessary requests for additional experiments in young animals which, as evidence has shown, are difficult to justify from a cost-benefit point of view. Additionally, recent innovations in personalised medicine for the identification of effective drug regimes (Berkers et al., 2019), use of adult clinical data for evaluating safe starting doses for children (Visalli et al., 2018) and refinement of in silico methods for pharmacokinetics (Smits et al., 2018) are all relevant and important methods with more direct applicability to drug development in neonates and children than the use of non-human animal models in JAS. This guideline could promote the use of advanced tools such as these within an integrated package, ensuring that JAS are considered as an absolute last resort. For example, Smits et al conclude that "PBPK [physiologically-based pharmacokinetic modelling is one of the tools to overcome the current limitations in neonatal drug development, with a proven track record in adults, and promising results in children". A recent article suggested that "publication of the rationale with details of why juvenile animal work is being proposed by a drug company or requested by the regulators" would be useful for a fully transparent debate on the case for JAS. (Baldrick, 2013).

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	We agree that this information should be made available to the public and we request that serious consideration is made to the conduct of a multi-national review on the true value of JAS to inform paediatric risk assessment. In the meantime, the use of JAS, especially multiple JAS studies in one or more species or those with multiple complex endpoints, should be discouraged. Some of the literature described above should be included in the guideline, even as recommended reading, to guide and better inform industry and regulators.
2	The document is well written with sufficient clarity on most topics. We appreciate emphasis on Weight of Evidence approach as the primary mechanism for determining the need for JAS. The ability to incorporate developmental ages in the chronic and/or PPND studies and measuring only relevant endpoints is appreciated. The examples provided in appendices for clarity and in support of the approaches mentioned in the document were very helpful. There are some ambiguities that have been included as specific comments below.
3	There are still quite a lot of redundancies both between chapters and between individual sections within chapters. Example: Lines 24-28: In three lines, there is three times the word “recommend(ation)”, and there are redundancies between 1st and 2nd sentence. Propose to avoid/delete repetitions/redundancies as much as possible to facilitate reading and understanding. See also “comments to wording” below.
4	This draft guideline recommends a core set of endpoints for all JAS, even if those endpoints are not focused on the concerns raised in the WOE assessment. We agree with this concept that if the need for a JAS is driven from a general lack of understanding of the pharmacology / mode of action then a more traditional approach with the core endpoints is appropriate. However, targeted JAS to address specific concerns may not always need all core endpoints (e.g., specific pediatric concern for a well-established mode of action). The guideline should be revised accordingly. See comments to lines 269-272 for more detail. This guideline should not only reflect the concept of whether JAS are warranted to support pediatric trials but also that JAS can be conducted concurrent to or after clinical trials for other purposes such as labelling. See comments to line 120 for more detail. The exclusions from the scope of this guideline are unfortunate as guidance is scarce and similar principles would apply. See comments to lines 103-104 for more detail. One of the key objectives of this guideline is harmonization of the approach whether JAS is needed or not (WoE approach). This should be consistently communicated throughout the guideline. See comments to lines 82 and 84 for more detail. The WoE text, the related Figures and Appendices require update and need to become aligned. See comments to lines 158 to 164 and lines 757 to 791 for more detail. The current proposed litter allocation text (3.9.1 and 3.9.2) is rather prescriptive. The text describes the singular litter allocation approach and can serve as an important example, yet this design should not be the only accepted approach. See comments to

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	lines 597 ff. for more detail.
5	The text of the S11 guideline proposal is well written and is provided with comprehensive material, appropriately complementing the already existing ICH guidelines that refer to juvenile animal studies. The text appropriately indicates that paediatric studies on drug safety and efficacy require substantially higher number of juvenile animals than the adult counterparts. One major issue in this particular field is the lack of standardized and universally recognized protocols. There is also no consistence in how preclinical studies should be described into published articles, and this leads to the impossibility to compare results among different studies and laboratories. This obviously makes it clear that the concept of data reuse is still far from being achievable. In our opinion, in order to optimize future efficacy and safety testing according to the 3R principles, and to increase the effectiveness of developmental studies, common data elements (CDEs) must be set and provided. The use of both core CDEs forms, and forms for CDEs specifically tailored for developmental studies (as the Appendix A) might help to standardize study design and make studies more transparent and cost/time-effective. One major goal should be the achievement of a future standardized system for the preclinical data collection, to be used to conduct meta-analyses for paediatric studies.

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes
44-46	3	**Comments:** Not all anticancer pharmaceuticals are in the scope of S9; the term "anticancer" is very general, rephrase or delete: and I propose to rephrase or to delete it. …those pharmaceuticals included in the scope of the ICH S9 guideline~~, i.e., anticancer ICH S9 guideline pharmaceuticals~~
52	3	**Proposed change:** Switch position: first pharmacodynamics, then pharmakokinetics.
55-67	3	**Comments:** In this chapter it would better to start with the information given in line 77-78, continue with paragraph 63 to 67 and then paragraph 56-62. So it would be clear that there should be a plan, followed by a WoE approach which then could results in JAS which could in turn be integrated in the "traditional" toxicology package.
71	4	**Comments:** clerical error (use of hyphen). **Proposed change:** Change to 'No observed adverse effect level' or 'No-observed-adverse-effect level'
80-87	6	**Comments:** The recommendation of international standards to promote harmonization of nonclinical safety studies in development of paediatric medicines will not only provide a basis for comparison of data among various regions but will also allow for compilation of larger data sets derived from multiple study sights. The creation of larger data sets will lead to more precise findings and relevant conclusions as to the safety and efficacy of paediatric pharmaceuticals. Furthermore, harmonization across various regions will be of significance in limiting the use of animal studies (JAS) to accomplish similar research end-points, avoid any overlap in investigation, and consolidate efforts and limited resources. Such initiatives can be realized through the utilization of paediatric research networks and other professional organizations.
82-84	4	**Comments:** The purpose statement should include harmonisation of the approach for nonclinical safety assessment recommended to support the development of paediatric medicines. The current wording implies only guidance for studies if

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		needed but the rationale for if a study is needed is just as important, as described in the scope (Section 1.3). Modification proposed **Proposed change:** Line 82, "…and promote harmonisation of, the nonclinical safety ~~studies~~ **assessment** recommended to support…" Line 84, "Harmonisation of the guidance for nonclinical safety ~~studies~~ **assessment** will define the current recommendations…"
89	3	**Comments:** Clarify if adult clinical investigation are meant
89-94	6	**Comments:** The need, timing, and design of JAS is the main focus of the ICH guideline S11 in order to avoid unnecessary use of animal studies as well as to insure maximum efficacy by selecting the appropriate time frames of animal development that most correspond to the period of human development in question, and which can vary significantly in terms of PK, PD, and organ maturation.
90-109	3	**Comments:** It should be added that distribution behaviour and tissue exposure are also important factors, see also chapter 2.3.3 lines 159 following
93	3	**Proposed change:** and nonclinical *in vitro* and *in vivo* animal**,** and clinical safety data (add comma)
93-94	1	"This guideline reflects current thinking based on collations of examples by regulatory agencies, by industry surveys, and literature". **Comments:** As mentioned above, it would be helpful to include some of the key resources that contributed to the guideline in the 'references' section.
94-95	3	**Comments:** Sentence appears redundant (see general comment). ~~A WoE approach considers multiple factors evaluated together and, 94 therefore, a single factor should not be considered in isolation.~~
96-104	6	**Comments:** The scope of the guidelines should be as stated limited to pharmaceuticals for consideration in the paediatric population such as anticancer drugs, with or without previous use in the adult population and other molecular therapies, i.e. gene therapy, tissue engineered products, or vaccines are excluded from

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		these guidelines.
99-102	1	"The ICH S9 guideline should be consulted for recommendations on whether to conduct JAS for those pharmaceuticals included in the scope of the ICH S9 guideline, i.e., anticancer pharmaceuticals. The ICH S11 guideline should be consulted for study design in all cases where a study is considered to be warranted". **Proposed change:** The ICH S9 guideline should be consulted for recommendations on whether to conduct JAS for those pharmaceuticals included in the scope of the ICH S9 guideline, i.e., anticancer pharmaceuticals **intended to treat patients with advanced cancer**. The ICH S11 guideline should be consulted for study design in all cases where a study is considered to be warranted.
100-102	4	**Comments:** sentence appears incomplete, addition proposed **Proposed change:** …in all cases, **including oncology indications,** where a study is …
103-104	4	**Comments:** addition proposed to improve clarity The exclusions from the scope of this guideline are unfortunate as guidance is scarce and similar principles would apply. Providing a rationale or clarification why certain modalities are excluded would be welcome (e.g., in a footnote). Also there is a lack of clarity that ICH S6 products are in scope, despite the use of a monoclonal antibody as an example case. **Proposed change:** **Both, small molecule therapeutics and biotechnology-derived pharmaceuticals as defined in ICH S6(R1) are within scope**. Although tissue engineered products, gene and cellular therapies, and vaccines are excluded from the scope of this guideline **similar principles can apply**.
106-110	4	**Comments:** The temporal relationship of developmental processes and drug exposure is the key difference between paediatric and adult patients. Proposed change: "Paediatric patients, who can receive medicines during periods of rapid growth and postnatal development of several organ systems, represent a distinct population compared to adults. Immaturity of organ systems in paediatric patients as well as maturation of systems during drug treatment can affect drug pharmacokinetics (PK), pharmacodynamics (PD), and/or off-target effects of medicines, potentially leading to differences in toxicity and/or efficacy profiles between paediatric and adult patients."

Line no.	Stakeholder no.	Comment and rationale; proposed changes
106-129	6	**Comments:** The general principle of paediatric growth as related to drug development is an important aspect that must be taken into consideration when determining the timing of clinical investigations, i.e. during prenatal or postnatal development, and to improve safety aspects. An understanding of paediatric growth development including important milestones will diminish the likelihood of unwanted interventions and alleviate the need for JAS studies. The use of WoE Factors as a guideline for determining if JAS studies are warranted takes into consideration such factors as patient age, organ development, pharmacologic target, and clinical treatment duration in determining if nonclinical studies should be undertaken. When many of these factors are affirmative, i.e. significant evidence exists then appropriate non-clinical studies such as JAS should be conducted. In order to avoid excessive waste of limited research resources such as funding and animal experimentation, the WoE approach should be carefully followed and any additional non-clinical studies justified based on necessity.
110	4	**Comments:** consider the use of 'and/or' **Proposed change:** …and **/ or** when compared to adults.
111	4	**Comments:** 'early' in the program/development **Proposed change:** use the term **timely** instead
111 (figure 1)	3	**Comments:** The clinical parameters (youngest intended patient age and dosing duration) set the scene for the WoE – they will help determine if there are potential adverse effects on developing organ systems. All three factors (youngest intended patient age, effects on developing organ systems and clinical treatment duration) should be highlighted as of highest importance
111 (figure 1)	3	**Comments:** Propose to change "modality of pharmaceuticals" to "selectivity of the pharmaceutical" and grading only high and low
116	4	**Comments:** 'earlier than usual' is not clear

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change: …earlier than **the normal drug development paradigm,** with modifications…
120	4	**Comments:** WoE assessment is not necessarily needed prior to each paediatric trial (e.g., according to ICH M3, no JAS is needed prior to a single dose PK study in children). Some JAS can be required to communicate potential safety risks in the label which cannot be addressed clinically. **Proposed change:** ~~Prior to each paediatric trial,~~ **To support paediatric development and marketing, …**
123	4	**Comments:** duration of treatment (or dosing) is missing **Proposed change:** …depending on paediatric age, indication **and duration of treatment**.
124-129	1	"The conduct of additional nonclinical investigations should be undertaken only when previous animal and human data are judged to be insufficient to support paediatric studies. JAS are designed to address identified safety concerns that cannot adequately be addressed in other nonclinical studies or paediatric clinical trials, including potential long-term safety effects. This guideline recommends a customized JAS that comprises core design elements and potential additional elements driven by specific concerns". **Proposed change:** The conduct of additional nonclinical investigations should be undertaken only when previous animal and human data, **pharmacological data and data from pharmacokinetic modelling/simulation systems** are judged to be insufficient to support paediatric studies. ~~JAS are designed to address~~ **If** identified safety concerns ~~that~~ cannot adequately be addressed in ~~other~~ nonclinical studies **such as in vitro and ex vivo investigations**, or **in adult or** paediatric clinical trials, ~~including potential long-term safety effects~~ **then a JAS may be considered as a last resort if scientifically justified. However, it should be noted that the value of JAS has not been fully elucidated and should therefore only be considered under rare circumstances and not as a default approach. Furthermore, the propensity for mortality to occur is generally higher in juvenile animals compared to adult animals and, in accordance with the 3Rs principles, their use should be avoided as much as possible.** This guideline ~~recommends~~ **describes** a customized JAS that comprises core design elements and potential additional elements driven by specific concerns.

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126-130	3	**Comments:** The criticality of the treatment duration is closely linked to the age of dosing initiation: the treatment of a neonate for 3 months is probably more critical than the treatment of a six year old for 1 year. Propose to discuss this in context with line 122-125.
131-132	2	**Comments:** For clarity, examples could be given for when *"clinical data are considered sufficient"* and *"safety concerns can be clinically managed"*. **Proposed change:** An example in parentheses behind each point would be helpful.
133-145	6	**Comments:** The clinical context of each individual paediatric subject must be taken into consideration and research designed according to the current needs of the patient. In cases with limiting existing data and severe debilitating diseases, which warrant immediate treatment, research should be conducted in a timely expedient manner in order to accelerate the delivery of safety data and facilitate the introduction of the pharmaceutical in question to the patient population in greatest need. A risk-benefit assessment should be made and utilization of JAS conducted in parallel to clinical investigation. The data obtained from animal studies will provide additional evidence as to safety including maximum tolerable dosages, minimal effective dosages, half life degradation, and overall toxicity. This may be especially applicable to oncological patients in the paediatric population, where only limited data may exist from adult studies.
137-139	4	**Comments:** Modifications to improve clarity. **Proposed change:** "**The need,** design and timing of ***any*** additional nonclinical investigations ***will depend on the identified*** safety concerns and the intended clinical use."
140-145	4	**Comments:** This paragraph is dis-jointed. Modification suggested. Proposed change: "For severely debilitating or life-threatening diseases, or diseases with serious unmet medical need in paediatrics, the sponsor and regulatory agencies should discuss the benefit of producing additional data versus the delay in patient access to the medication caused by additional nonclinical testing. The decision regarding the need for and timing of nonclinical testing should be based on a careful and cautious risk-benefit evaluation. If a safety concern is identified for further clinical development, appropriate nonclinical studies (e.g., JAS) should be considered, and could be

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		conducted in parallel with paediatric clinical investigation."
143-145	1	"If a safety concern is identified for further clinical development, appropriate nonclinical studies (e.g., JAS) should be considered, and could be conducted in parallel with clinical investigations". **Proposed change:** If a safety concern is identified for further clinical development, appropriate nonclinical studies (e.g., **in vitro or ex vivo investigations** ~~JAS~~) should be considered, and could be conducted in parallel with clinical investigations.
147-169	6	**Comments:** No additional comments to this section- the WoE factors approach is well explained taking into consideration multiple clinical and non clinical factors in the decision making process of whether or not to utilize JAS.
148-153	3	**Proposed change:** Propose shortening to avoid redundancies (see general comment).
151-153	4	**Comments:** Existing text stating whether studies are warranted is vague and not useful if the studies would not address the specified concerns. Suggest revising to state clearly that additional nonclinical studies should be considered only if they would address the specified concerns. **Proposed change:** "…whether additional nonclinical studies ~~are warranted~~ **would address those concerns**."
154-155	4	**Comments:** Modification to improve clarity. Pertinent new safety information should also be considered. **Proposed change:** The WoE evaluation should be conducted when designing the initial paediatric **clinical** development plan, but ~~revisited~~ **reassessed** if there are changes in age ranges, **treatment duration** and/or indications **or pertinent new clinical or nonclinical safety information**."
157-158	3	**Comments:** Clarify what is meant with "identified "Proven? Based on data?"
158-159	4	**Comments:** Modification to improve clarity. **Proposed change:**

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		The individual factors are presented ~~below~~ on the left of ~~the~~ Figure **1**.
159-161, 166-168	4	**Comments:** Modification to improve clarity. **Proposed change:** The most important factors are the youngest intended patient age, nonclinical or clinical information from previously conducted studies, and whether there are ~~known~~ ~~(or~~ suspected~~)~~ adverse effects on developing organ systems of **paediatric clinical trial subjects**. ~~the~~ ~~patients~~ ~~during the conduct of the paediatric trial~~.
159 and 168	4	**Comments:** The factor “effects on developing organ systems” listed in Fig. 1 should be modulated with the relevance of JAS to detect such effects. It is suggested that further consideration be given to the translatability and biological relevance of the JAS data to humans. **Proposed change:** L159 and L168 The most important factors are the youngest intended patient age and whether there are known (or suspected) adverse effects on developing organ systems of the patients during the conduct of the paediatric trial. **In addition, the translatability and biological relevance of the JAS data to humans should be considered**.”
159-169	3 SMC	**Comments:** Tissue distribution data and tissue exposure should be added and discussed
161 and 168	4	**Comment:** Modification suggested. **Proposed change:** “The other factors are not listed in order ~~of weight in the figure~~ **of importance**.“
163	4	**Comments:** “clinical management” is not a broadly understood term **Proposed change:** add/define term ‘clinical safety management’ in glossary
163-169	5	**Comments:** Figure 1 efficiently summarizes the most relevant factors and the gradient of weight when considering the need of non-clinical safety investigations. We however think that the first row that considers the “Youngest intended patient age” as one of the most impactful factors in the rational of performing further

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		preclinical studies, does not fully represent the variety of developmental peculiarities that distinguish each paediatric age from an adult. These ages are indeed very specific and represent a characteristic pattern of hormonal and developmental status which differs quite strongly from the one encountered in adults. Numerous experimental studies on behavioural toxicity in developing animals identify indeed the adolescence as another critical developmental period, with increased risk for adverse drug effects. Both transient and long-lasting changes in CNS functions were described after exposure of adolescent animals to various neuroactive drugs (for example Andersen and Navalta, 2004). **Proposed change:** The scale of "Youngest intended patient age" could be extended, ranging from neonates to at least 18Yr rather than 12+Yr. Adolescence as intended developmental patient stage should be mentioned among important WoE factors.
164 (Fig. 1)	4	**Comments:** The blue shading of the first two WoE factors is not well understood. **Proposed change:** suggestion to delete color difference
164 (Fig. 1), 177	4	**Comments:** WoE factors/bubbles would be easier to follow in section 2.3 if the same terminology was used. And there seems to be a conflict between Fig 1 (legend indicates youngest patient age and known/suspected adverse effects on developing organ systems are the most important factors) and text in 2.3.1 (indicates that the established efficacy and safety profile are the first point to consider). **Proposed change:** Revise Fig 1 to match 2.3 text
164 (Fig. 1)	4	**Comments:** 'Modality of Pharmaceutical' is not the correct term **Proposed change:** Change to **'Risk for off-target effects'** (High - Low)
164 (Fig. 1)	4	**Comments:** Modifications to improve clarity. **Proposed change:** **"**Adult Nonclinical **Data** Only"; Adult Clinical **Data**; P**a**ediatric Clinical **Data**"

Line no.	Stakeholder no.	Comment and rationale; proposed changes
165-169	2	**Comments:** Clinical PK modelling is discussed as a relevant option for predicting paediatric exposures, but actual clinical PK data in pedes is not discussed (nor is it mentioned in Section 2.3.1). **Proposed change:** ICH M3 states that JAS is generally not required for short term PK studies in paediatric populations. It would be helpful if this section touched on the value of short term paediatric PK data in the WoE approach.
168	3	**Comments:** For the follow-up of differences between paediatric and adult patients based on PK modelling and simulation, nonclinical investigations e.g. in vitro are proposed. Could you give examples for in vitro studies or explain rationale?
172-174	5	**Comments:** What is written is correct. We highlight the fact that the perspective of this statement is related to the general approach of drug development which repurposes drugs already developed and designed for adults or young adults. The position of EPTRI consortium in this sense is that this general mentality should change, and the concept of drugs which are specifically designed for the youngest paediatric population should be given a new consideration.
172-188	6	**Comments:** In essence, when assessing the need for additional Juvenile Animal Studies (JAS), the safety profile and efficacy of the pharmaceutical agent in question must be considered as well as the age of paediatric population in question. Younger subjects are more vulnerable to any form of intervention and the duration of exposure is also a significant factor with longer exposure increasing risks of adverse effects. The risk-benefit of obtaining data versus putting the population in question at risk must be assessed and resorting to JAS taken into consideration.
177	4	**Comments:** Modifications to improve clarity. **Proposed change:** "...~~at the lower end of the age range~~ **to support younger paediatric age ranges**.
179-182	4	**Comments:** Modifications to improve clarity. **Proposed change:** "Longer durations of treatment are more likely to expose a paediatric subject

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		during a developmentally sensitive window, whereas short-term use of a pharmaceutical is less likely to affect some aspects of development such as growth. Long duration of use is therefore more likely to warrant further nonclinical studies than short-term treatments."
183-184	5	**Comments:** The fact that non-clinical studies are not warranted when existing clinical data are considered sufficient to support paediatric use is still subjective, as the aspects considered to define such data "sufficient" do not (and probably at this stage cannot) consider the totality of the physiological impacts that a drug can have on a paediatric individual.
Section 2.3.2.	1	**Comments:** An important element that is missing from this section is the consideration of effects from other compounds from the same pharmacological class or with similar safety profiles or pharmacological activity as the target drug. According to the available literature, this data can also be a useful factor for informing the WoE evaluation (Carleer & Karres, 2011).
190	4	**Comments:** need to clarify 'secondary pharmacological properties'. Secondary pharmacology can include both potential off-target as well as unintentional on-target effects (also relevant for pharmaceuticals with high selectivity). In this context here, probably only off-target effects are meant (see also L204: 'secondary pharmacodynamic effects' …) **Proposed change:** **Pharmacological properties** of a … **Proposed change:** Define 'secondary pharmacology' in glossary (off-target only)
190-191	3	**Comments:** However, if ~~there is an identified~~ safety concern **was identified in the PPND study** ~~that could lead to effects on postnatal development~~
190-201	6	**Comments:** As the selectivity of pharmaceuticals can vary with some drugs acting on a specific set of targets or receptors with sophisticated mechanisms of actions and minimal side effects, other pharmaceuticals may be less specific and more generally acting with multiple effects on various organ systems. As a result with increased side effects as well and hence, such drugs may warrant the use of JAS. In essence, the pharmacology of each agent in question must be understood in terms of mechanism of action, metabolism, potential adverse effects, and of course, taking into consideration physiological and

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		developmental differences between children and adults.
195-197	4	**Comments:** Addition to emphasize that effects in homozygous null animals (absence throughout in utero development) should not be over-interpreted as data from can be substantially different from potential effects of postnatal pharmacological inhibition. **Proposed change:** …may also identify **in utero** developmental effects of potential **relevance** ~~concern~~ for…
198-200	4	**Comments:** Modifications to improve clarity **Proposed change:** If the known **pharmacologic target of a pharmaceutical** ~~pharmacology of a medicine~~ has the potential to impact the development of the intended paediatric population, or the role of the ~~pharmacology~~ **pharmacologic target** on development is not understood or reasonably predictable, further nonclinical investigations should be considered.
199-200	3	**Comments:** This is also true for PPND studies, not just ePPND.
201	4	**Comments:** Given that some New Chemical Entities can also be highly selective (against multiple other targets), it is proposed that the brackets *(e.g., monoclonal antibodies)* are removed as it could be perceived to mean that only large molecules are highly selective. **Proposed change:** "Potential adverse effects of pharmaceuticals with high selectivity for their target ~~(e.g., monoclonal antibodies)~~ are more likely to be related to exaggerated pharmacology and therefore be more predictable than effects of pharmaceuticals with lower selectivity for their pharmacologic target."
207-213	3	**Comments:** Mostly repetition, propose to remove sub chapter
209	4	**Comments:** Modification to improve clarity Does the term '*underlying pharmacology'* include data from similar compounds from the same pharmacological class? The text in ICH M3 (R2) at least does '*including effects from other drugs of the pharmacological class'.*

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Would it therefore be adequate to cite such existing data (but 'right of reference or use' can make this impossible in US)? **Proposed change:** Further nonclinical studies might not add value when ~~the underlying pharmacology has~~ **studies with other drugs of the same pharmacological class have** already identified a particular paediatric hazard.
216-217	1	"The use of clinical PK modelling and simulation systems for the purpose of predicting PK/ADME characteristics in paediatric populations can be more relevant than conducting JAS. If the results of the PK modelling and simulation indicate that there will be significant differences between adult and paediatric populations, then nonclinical investigations (e.g., in vitro studies) can be helpful to determine the potential impact of these differences on toxicity". **Proposed change:** **In most cases,** the use of clinical PK modelling and simulation systems for the purpose of predicting PK/ADME characteristics in paediatric populations **is** ~~can be~~ more relevant than conducting JAS. If the results of the PK modelling and simulation […]
217-220	4	**Comments:** Modification to improve clarity **Proposed change:** "…simulation indicate ~~that there will be~~ **likely** significant **exposure** differences between adult and …"
222-250	6	**Comments:** Data obtained from animal studies as to drug toxicity should be taken into strong consideration when designing paediatric investigations. The specific effects on organs and tissues at various ages and stages of development may vary and must be carefully assessed in order to avoid potential toxicity to immature organ systems such as liver and kidneys involved in bioavailability and metabolism of pharmaceutical agents. Adverse effects found in multiples species of animals can also indicate a significant degree of toxicity and a safety concern for the paediatric population.
226-227	4	**Comments:** It is stated that safety signals in more than one species are of increased concern. This is not always the case if there is a (human relevant) biological rationale for why it was only observed in one species. **Proposed change:**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		"Safety signals that occur in adult animals of more than one species are **more likely to be** of increased concern."
236	4	**Comments:** Modification to improve clarity **Proposed change:** If PPND/**ePPND** study data are…
237/ whole docum ent	3	**Comments:** What is the difference between a preliminary and a DRF? In line 237 DRF is an example for a preliminiary study, later in the document it says preliminary study or DRF (e.g. line 249 or 438). The difference is never spelled out. Please clarify.
253-256	4	**Comments:** Addition to improve clarity **Proposed change:** …not be informative or warranted. **If an additional nonclinical study cannot be designed, conducted, or interpreted that would inform paediatric patient safety then it should not be conducted.**
253-256	4	**Comments:** '…acceptable systemic exposures in the range of those expected in paediatric patients…" is assessed in DRF **Proposed change:** refer to Section 3.2 (DRF/PK studies)
253-256	4	**Comments:** Exposure in JAS may not always have to be in the range of paediatric patients as long as the pharmacologic target is saturated in JAS. **Proposed change:** If a study in animals cannot be conducted with dose levels that provide acceptable systemic exposures **or relevant target engagement** in the range of those expected in paediatric patients, …
254	4	**Comments:** Exposure is more relevant than dose (dose adjustments can be required or neutralizing anti-drug antibody may interfere with acceptable exposure at otherwise appropriate dose levels) **Proposed change:** If a study in animals cannot be conducted ~~with dose levels that provide~~ **at**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		acceptable systemic exposures in the range of…
258-263	6	**Comments:** As previously stated the WoE approach should be closely adhered to when evaluating the need for JAS. The criteria should be strictly applied in order to avoid unnecessary use of financial resources as well as indiscriminate use of animal subjects.
260-262	1	"When a study is warranted, the specifics of the identified safety concerns will define the objectives of the nonclinical investigation; this could be a JAS or another study (e.g., in vitro or ex vivo investigations)". **Proposed change:** "When a study is warranted, the specifics of the identified safety concerns will define the objectives of the nonclinical investigation; this could be **an in vitro or ex vivo investigation or, under rare circumstances,** a JAS. ~~or another study (e.g., in vitro or ex vivo investigations).~~ ***This would then lead into a new section that covers the design of in vitro and ex vivo studies, as suggested in our general comments above. For example, more information on the use and design of biosimulation studies should be provided e.g. physiologically-based PK models from in vitro-in silico data, which have proven to be a consistent and reliable evidence-based approach to optimise clinical trial design and inform the drug label for paediatric medicines (Marier et al. 2016, Zhao et al 2014). This section should also provide guidance on the use of other in vitro models (e.g. in vitro gastrointestinal tract models to study drug bioavailability in children) and ex vivo models (e.g. use of tumour cells and biopsies) to support paediatric drug development.***
263	4	**Comments:** clerical error **Proposed change:** Appendix B is referred to before Appendix A. Text or appendices should be reorganized accordingly.
264-274	3	**Comments:** Suggest to re-arrange the order: start with information on pharmacological or toxicological target (bullets 1 and 3), then PK/TK (bullets 2 and 5) and then feasibility
266-282	6	**Comments:** This section states the importance of study design in terms of addressing specific organ systems and general safety. End points must be clearly defined in order to avoid misinterpretation of data and obtain statistically significant

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		conclusions. The population sample size should be large enough to add confidence and validity to the results. Nevertheless, an understanding of organ development, age, maturation, toxicity, animal species, etc. is of the essence in order to achieve clinically relevant and statistically significant results.
267-269	1	"This section contains recommendations on study design considerations, core endpoints to be included in all studies, and additional endpoints that can be included to address specific concerns. A JAS design including all potential endpoints is not recommended without rationale". **Comments:** A recent review (described in the general comments section above) found that, of the small proportion of JAS that revealed novel toxicities, "the elucidation of the toxicities was accomplished using routine toxicological assessments and not as a consequence of performing a large complex study with every possible endpoint monitored, as seems to be the current trend" (Bailey & Marien, 2009). The authors express their concern that "investigators are continually being requested to perform bigger and more complex studies" without any proven benefit or evidence that these more sophisticated and complex study designs actually generate any meaningful results. Other authors have also warned against "inappropriate or unnecessary studies being performed or the inclusion of parameters, which generate little or no useful information" (De Schaepdrijver et al., 2008). We therefore suggest that more effort is made to stress that the use of the described 'additional endpoints' should be limited to very rare situations only. **Proposed change:** This section contains recommendations on study design considerations, core endpoints to be included in all studies, and additional endpoints that ~~can~~ **may, under rare circumstances,** be included to address specific concerns. A JAS design including all potential endpoints **by default** is not recommended ~~without rationale~~.
269	4	**Comments:** Modification to improve clarity. Addition to address if the reason to conduct a study is driven by a specific, identified safety concern and that study design should be customized to address that concern then it may not be appropriate to include "Core endpoints" as described in 3.8.1. **Proposed change:** ~~A JAS design including all potential additional endpoints is not recommended without rationale.~~ **The overall design of the JAS, including proposed non-core endpoints, needs to be justified. Similarly, a targeted JAS addressing specific concern may not necessarily include all core endpoints, if justified.**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
270-272	4	**Comments:** Modification to improve clarity. **Proposed change:** If the reason to conduct a ~~study~~ **JAS** is primarily driven by a ~~specific, identified~~ safety concern ~~for paediatric patients,~~ **that cannot be addressed with existing data,** the ~~study~~ **JAS** design should be ~~customized to address particular aspects of~~ **focused on** function**al** or development**al** ~~of a target organ or system of~~ **endpoints that address the** concern.
272-274	4	**Comments:** Modification to improve clarity. **Proposed change:** If the rationale to conduct a ~~study~~ **JAS** is based on a concern for patient safety due to lack of relevant knowledge of the **pharmaceutical's** ~~pharmacology~~ **pharmacologic effects**, the study design would generally be ~~broader~~ **a core study** ~~and include~~ **with** additional endpoints as appropriate
274	3	**Comments:** Please clarify if similarity to human ADME characteristics is meant; proposal: Similary ~~of~~ **to human** ADME characteristics What is the difference to bullet point 2?
280	4	**Comments:** Delete 'quite' **Proposed change:** … and/or regulation of maturation can be ~~quite~~ different between humans and animals.
281	2	**Comments:** For clarity, an example could be given of a rare case in which an NHP JAS was required. **Proposed change:** An example in parentheses behind the statement would be helpful.
282-283	3	**Comments:** Should this approach only be considered when the data in adults were also generated with a homologous protein? Please clarify.
283-303	5	**Comments:** Preclinical data on possible age-related differences in pharmacological efficacy can help substantially with selection of optimal dose range for safety tests.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Many tests used for preclinical drug safety screening in adult animals were modified for immature rodents and they could be used as a part of preliminary studies.
287	4	**Comments:** Addition to improve clarity. **Proposed change:** …in a definitive JAS. **DRF/PK studies typically have limited endpoints and not necessarily expected to include all core endpoints (e.g., pathology).**
287-289	3	**Comments:** This paragraph should be moved to chapter 2.3.2.
290	4	**Comments:** DRF study design should be kept flexible and as needed **Proposed change:** ~~The DRF dosing period generally lasts a few weeks, e.g., typically until shortly after weaning in rodents.~~
291	4	**Comments:** Modification to improve clarity. **Proposed change:** ….adults and juveniles, ~~a second~~ **an additional** DRF study…
296	4	**Comments:** Modification to improve clarity. **Proposed change:** ….at **anticipated paediatric** ~~clinically~~ ~~relevant~~ systemic ….
298-302	3	**Comments:** Re-consider length of the sentence.
299-300	4	**Comments:** We are concerned that these hypothetical examples may become requirements whenever there are differences. **Proposed change:** …. additional investigations ~~(e.g., assessment of protein binding values or blood-brain barrier penetration)~~ can be useful…

Line no.	Stakeholder no.	Comment and rationale; proposed changes
305	4	**Comments:** Why is rat preferred over mouse? **Proposed change:** In principle, the ~~rat~~ **same species as used in adult repeat-dose studies** should initially be considered as the species for a JAS.
305-335	6	**Comments:** In general, the selection of animal species should be based on existing data regarding the efficacy and suitability of the species for the particular aims and objectives of the study. Primary and secondary endpoints should be clearly defined as well as anticipated outcomes. Certain species of mice are better models depending on the type of investigation in question such as anticoagulants, reproductive drugs, and dermatological or oncological agents. Furthermore, the behaviour effects of the experimental animals can vary across species; hence, careful and timely selection of the experimental model is of utmost importance.
307-308	4	**Comments:** Addition to improve clarity. Pharmacological relevance is a critical factor in choosing a nonclinical species. **Proposed change:** "In all cases, the selected species should be justified, as nonclinical studies in a **pharmacologically** non-relevant species can give rise to misinterpretation and are not recommended."
309-320	1	**Comments:** The main "factors for consideration when selecting an appropriate species" for JAS are listed here. Ethical and animal welfare considerations are missing from this list and should be added to further promote the importance of the 3Rs. **Proposed change:** Add the following bullet point to the list: '**Ethical and animal welfare considerations of conducting the study in the selected species**'.
323-327	1	"While for biopharmaceuticals NHPs are pharmacological responders in many cases, the conduct of JAS in NHPs is challenging for both scientific and practical reasons. There is limited added value of performing JAS in younger NHP as compared to the 2-4 year old NHP used in general toxicity studies and, therefore, alternative approaches to obtaining the necessary data are encouraged. Only in rare cases is the value of JAS conducted in NHP justifiable". **Comments:**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		We appreciate that the use of NHPs in JAS is not recommended by the guideline. However, based on the available literature, the use of dogs and rodents in JAS is also of questionable value and should therefore be discouraged. As described above, a review study found that 85.7% of the results generated from JAS in puppies, and 75% of the results from JAS in rat pups, could have been predicted by pharmacology or adult toxicity data (Bailey & Marien, 2011). As well as being predictable, results in JAS using dogs have also been shown to be unreliable. For example, "quinolones affect the cartilage of young dogs. This resulted in broad warnings against the use of quinolones in children. Were these warnings justified? For paediatric clinicians quinolones are important reserve antibiotics" (Rose, 2011). Due to insurmountable species differences between the development of puppies and human children, one article concluded that "the dog is unsuitable in so many ways that it is difficult to many any case for its use in juvenile studies" (Downes, 2012). **Proposed change:** While for biopharmaceuticals NHPs are pharmacological responders in many cases, the conduct of JAS in NHPs is challenging for both scientific, ~~and~~ practical **and ethical** reasons. There is limited added value of performing JAS in younger NHP as compared to the 2-4 year old NHP used in general toxicity studies and, therefore, alternative approaches to obtaining the necessary data are encouraged. Only in rare cases is the value of JAS conducted in NHP justifiable. **Similarly, while dogs are often used as the second non-rodent species in general toxicology studies, there are substantial developmental differences between dogs and humans, which limits the added value of performing JAS in puppies.**
324-326	4	**Comments:** Addition to improve clarity. There are instances when the age of the NHPs used in toxicology studies exceeds 4 years. **Proposed change:** …younger NHP as compared to the 2-4 year old NHP **generally** used…
326-327	4	**Comments:** There have been a significant number of JAS NHP studies conducted recently at the request of the HAs. **Proposed change:** add example for 'rare case' to limit study calls
328-329	4	**Comments:** Modification to improve clarity. **Proposed change:**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		~~Consistent with ICH S6, a~~ **A** homologous protein, ~~when available,~~ **as detailed in ICH S6,** can be considered for the purposes of hazard identification in the rodent or other non-rodent species.
330	3	**Comments:** Dose Selection regarding dose adjustment as an alternate strategy **should** to be considered in this situation
330 onwards	4	**Comments:** Clerical errors. **Proposed change:** Use hyphen uniformly in 'paediatric-first' and 'paediatric-only'.
330-332	4	**Comments:** Modification and alternative placing. **Proposed change:** JAS in two species ~~would~~ **can** be warranted… Consider to move to L262 (i.e. WoE outcome)
332	4	**Comments:** Addition to improve clarity. **Proposed change:** **The conduct of a JAS in a second species to confirm findings in the first species is not warranted.** Consider to move to L262 (i.e. WoE outcome)
333-335	4	**Comments:** While it is possible for models of disease to provide useful safety information as part of the WoE, it can be difficult to interpret such studies for the purposes of human risk assessment. Addition to improve clarity. **Proposed change:** "**JAS using models of disease should not be conducted solely for safety assessment unless they can be clearly interpreted and useful for human risk assessment."**
337	4	**Comments:** Modification to improve readability. **Proposed change:** The age of **animals at** dosing initiation ~~in animals~~ should developmentally correspond to the youngest age of the intended paediatric population, ~~which~~ **and** will depend on a human-to-animal comparison of developmental periods

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		of organ system(s) of toxicological concern.
337-379	6	**Comments:** In general, the age of dosing of experimental animals should correspond to the developmental period in question of the paediatric population. Such correlations are often difficult to determine, and justification should be based on existing scientific evidence. (Appendix A) Furthermore, the dosing period and duration of the experimental animals should be defined and correlations with human paediatric subjects in terms of organ development and the desired effects in question made clear.
343-347	4	**Comments:** Addition to improve clarity. **Proposed change:** When determining the duration of administration in JAS, it is important to consider the **paediatric** age range and the shorter developmental period of animals compared to humans,…
344 and 356	3	**Comments:** Propose to move reference to note 1 to line 356.
348-350	4	**Comments:** Modification to improve clarity. **Proposed change:** The dosing period in JAS is not only defined by the ~~paediatric age stages~~ **intended paediatric age range** (e.g., > 2 years) ….
352-354	3	**Comments:** Is this sentence needed in this context?
355-360	4	**Comments:** Appendix A shows 12 years in human corresponds to less than 6 weeks old in rats, i.e. a 3-week dosing period (PND 21 to 42) would be sufficient to cover human age up to 12 years. Modification to improve readability. **Proposed change:** For example, to include the youngest intended patients of 2 years old up to patients 12 years of age with a clinical dosing duration of 14 days, the JAS can have a dosing period **of approximately 3 weeks** ~~longer than 14 days to incorporate exposure at all developmental stages corresponding to human patients from 2 to 12 years old (e.g., in the rat this would be approximately 6 weeks dosing duration, roughly~~ (postnatal day (PND) 21 to 42, See Appendix A).

Line no.	Stakeholder no.	Comment and rationale; proposed changes
355-360	5	**Comments:** The chapter 3.4 describes possible approaches on how to define the dosing time for the performance of safety JAS with respect to the developmental age range of the intended paediatric population the drug will be addressed to. In the reported example, authors claim that it is necessary to incorporate all age groups of rats that correspond with paediatric patients 2-12 years of age. Tested drug has to be administered for 6 weeks between P21 and P65. At least regarding brain development (for example Clancy et al, 2007), human new-borns or infants are comparable with P10-12 rats and adolescence starts around P35 and sexual maturation of male rats (at least Wistar) is finished around P50. The recommended testing interval lives therefore out the youngest age groups and, on the other hand, covers more mature developmental stages including puberty. Indeed, developmental extrapolation is to a certain extent organ-specific and this should be specified in the text.
361-362	4	**Comments:** The statement “as these species mature over a period of a few to several months…” is inconsistent with Figure A.3, for the dog, which shows puberty over a period of up to 12 months. **Proposed change:** either exclude dog in this phrase or adapt for consistency with Fig A3
366-367	3	**Comments:** Is this contradicting the statement in line 316-317? Or should “non rodent” be replaced by “NHP”?
366-369	4	**Comments:** Addition to improve clarity. **Proposed change:** … at different ages). **If subgroups with different dosing periods are used, all subgroups may need to be followed through to maturity to detect late effects.** This approach…
372	4	**Comments:** Addition to improve clarity. **Proposed change:** The benefits of this approach should be considered **along** with the drawbacks,…
380 ff.	4	**Comments:** The term “off-treatment period” is less well defined than the term ‘post-dosing period’. Off-treatment can also refer to the dosing interruption during an

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		intermittent dosing regimen (e.g., on-off treatment in oncology). Likewise, the term 'dosing' is preferred over 'treatment' as the latter can include interventions other than drug administration. **Proposed change:** consider to use "post-dosing period" throughout the entire document
386	4	**Comments:** clerical error (use of hyphen). **Proposed change:** Standardize "~~off-treatment~~ post-dosing" (or "~~off-treatment~~ post dosing") throughout the entire document.
394-398	3	**Comments:** What is the expectation concerning the availability of TK data to support dose adjustments? Are they in addition to the requirement in line 437?
398	4	**Comments:** addition of punctuation mark **Proposed change:** …considered.
401-402	3	**Comments:** Each additional endpoint (see Section 3.8.2) should ~~be considered and~~ justified to address an identified safety concern (Note 2).
401-402	4	**Comments:** "behavioural assessment" is too vague since several specific behavioural assessments can and are evaluated prior to "maturation". **Proposed change:** …expected to be reached (e.g., **learning and memory**~~behavioural assessment~~, immunological response in T-cell-dependent antibody response [TDAR]). Suggest providing a specific type of behavioural assessment that would fit this example.
403	3	**Comments:** The term "it is important" is very strict. Propose to weaken this a bit. The concurrent control group is still the most important for comparison and data interpretation.
405	4	**Comments:** Modification to improve clarity.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change: …the clinical population is only the ~~very young~~ lowest age ranges. Consider to refer to E11(R1) or
408-410	4	**Comments:** Modification to improve clarity. Not all non-rodent species have too long development time and are too variable (see section 3.4 line 361-362: minipig, and rabbit mature over a period of a few to several months, and with relative consistency). **Proposed change:** In non-rodents, **depending on the species,** the addition of post-treatment groups for JAS can be less useful due to the more…
		Comments: Modification to improve clarity. **Proposed change:** …variability, and fewer and less well ~~characterized~~ **established** assessments available to identify delayed or altered development **(e.g., learning and memory testing)**.
412-420	6	**Comments:** Whatever route of administration is chosen, i.e. IV,SC,PO,IM; a systemic effect should be achieved. Depending on the types of pharmaceuticals being utilized, i.e. antibiotics, chemotherapeutics, vaccines, etc. particular routes of administration could be more beneficial in terms of the achieved effects and duration, i.e. slow release with IM, by pass liver with PO, prolonged with I.V., etc.
426	4	**Comments:** Addition to improve clarify. **Proposed change:** Body weight loss or lack of **weight** gain during rapid growth periods….
428-430	4	**Comments:** Why is the low dose specifically identified? PK and/or tolerability can lead to that only the high-dose in JAS produces an exposure comparable to the intended paediatric population. **Proposed change:** ~~The low~~ **At least one** dose should ~~preferably~~ result in exposure levels similar to the anticipated…

Line no.	Stakeholder no.	Comment and rationale; proposed changes
432-433	2	**Comments:** This does not address target organs identified in toxicity studies of adult animals. It could be considered adding a precision. **Proposed change:** *"Histopathology should be performed on major organs (e.g., bone, brain, ovary, testis, heart, kidney, liver),* ***target organs identified in adult toxicity studies, and organs*** ~~*and those with*~~ *macroscopic lesions"*
432-433	3	Representative organs from digestive system, respiratory system, immune and endocrine systems are missing. **Proposed change:** "Histopathology should be performed on major organs (e.g., **adrenals,** bone **with bone marrow**, brain, ovary, testis, heart, kidney, liver**, lung, stomach, small and large intestine, spleen, thymus, thyroids, pituitary**) and those with macroscopic lesions".
436-440	3	**Comments:** Please clarify if additional TK are needed when dose adjustments are done (see comment lines 394-398)
441-443	1	"Each JAS should include the core endpoints defined in Section 3.8.1 below, unless justified otherwise. Each additional endpoint (see Section 3.8.2) should be considered and justified to address an identified safety concern (Note 2)". **Proposed change:** Each JAS should include the core endpoints defined in Section 3.8.1 below, unless justified otherwise. **In rare circumstances**, ~~Each~~ additional endpoint**s** (see Section 3.8.2) ~~should~~ **may** be considered ~~and justified~~ to address an identified safety concern. **However, the inclusion of each additional endpoint must be scientifically justified, and a rationale provided for how the results are expected to add value to the risk assessment** (Note 2).
441-445	6	**Comments:** Each endpoint should be in accordance with a rational clinical approach and justified based on questions seeking to be answered without compromising patient safety or in the case of JAS without causing excessive harm to the animal study group.
448-453	6	**Comments:** Physical examinations should be performed on the animal population throughout the study period not only to determine specific experimental effects but also to assess overall physical well-being, side effects, and observe behavioral changes, i.e. stress levels, mating behavior, maternal nursing, etc.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
450	4	**Comments:** addition of punctuation mark **Proposed change:** …treatment.
455-457	4	**Comments:** It would be helpful if more guidance was provided with regard to how frequently and for how long body weights should be measured (on average), as this can vary widely based on species. Addition to improve guidance. **Proposed change:** …should **be assessed at intervals appropriate for** ~~frequently recorded to~~ inform**ing** dose calculations.
455-458	6	**Comments:** The assessment of growth by measuring body weight is important in order to calculate appropriate dosages of the pharmaceutical agent in question.
460	6	**Comments:** Food consumption should be recorded and assessments made based on the species of animals used in the experimental model.
462-464	4	**Comments:** Onset of puberty should also be recorded when the post-dosing period encompasses the relevant developmental window. Addition to improve guidance. **Proposed change:** …are **generally** recommended when the ~~treatment period~~ **study design** encompasses the relevant developmental window.
462-64	6	**Comments:** When the study covers the early developmental period of the animal models, observations concerning sexual development should be made. Knowledge of when such changes occur as well as whether menstruation actually occurs or not, i.e. Sloughing of endometrial lining, is paramount as this can vary across animal species.
466 and 497	4	**Comments:** clinical chemistry can be done in plasma or serum. Modification suggested. **Proposed change:** … (~~serum~~ **clinical** chemistry and haematology)…

Line no.	Stakeholder no.	Comment and rationale; proposed changes
472	3	**Comments:** Since the development of the human eye is not listed in table A1 , please provide information when retina and optic nerve in humans are developed.
472-473	4	**Comments:** Histopathology should include target organs identified in adult toxicity to facilitate some of the comparisons recommended in the guideline. Addition to improve guidance. **Proposed change:** …and those with macroscopic lesions. **Histopathology should include target organs identified in the adult toxicity studies.**
473-474	4	**Comments:** The term “qualitative evaluation” is unclear (staging versus stage aware). The current text can also be interpreted that all pivotal studies would have testicular histopathology in mature animals. Since not all pivotal JAS complete at a mature age we suggest this sentence is deleted or re-worded (‘interpretation of testicular histopathology can be compromised if evaluated in immature animals’) **Proposed change:** remove last sentence
479	4	**Comments:** Incomplete guidance. Addition suggested. **Proposed change:** …timepoints of sample collection. **The TK assessment should consider both, parent compound and relevant metabolites.**
482	4	**Comments:** Modify to use terminology consistent with that used elsewhere in the document and under ICH. **Proposed change:** For ~~protein therapeutics~~ **biopharmaceuticals**, samples for anti-drug antibodies should be collected…
484	3	**Comments:** …identified in the W**o**E evaluation.
486	3	What is the difference between extension of pharmacology and developmental neurotoxicity?
486-	6	**Comments:**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
489		In addition to height and weight, long bone length, crown rump length, body length can be used to determine specific endpoints.
487	3	**Comments:** Cessation of treatment)**,** or both. **(add comma)**
487-489	4	**Comments:** Modification to use more general terminology. **Proposed change:** …length using ~~ultrasonic echo or X-ray~~ **appropriate imaging techniques** can be appropriate…
491-495	4	**Comments:** If more detailed skeletal evaluations are warranted, the endpoints should be selected based on the strength and nature of the concern, and the species used. **Proposed change:** When there is an identified concern about bone metabolism or structure, ~~the measurements of bone-related biomarkers and/or expanded histopathology (e.g., histomorphometry) ,~~ **additional skeletal endpoints** should be considered. ~~Assessment of bone mineral density (e.g., microdensitometry, dual energy X-ray absorptiometry, peripheral quantitative computed tomography [CT]) or bone structure (e.g., micro CT) can also be conducted as appropriate.~~ **The endpoints should be based on the st** **strength and nature of the identified concern and the test species involved.** Examples include assessments of bone mass and geometry using densitometric techniques, serum and urinary biomarkers of bone formation and resorption, and bone histomorphometry.
492-496	3	**Comments:** The modified Irwin test and the functional observational battery are both tests for effects on behavioural function in rodents. The other listed parameters (e.g. locomotor, coordination etc.) are endpoints. Please clarify and discriminate endpoints from test systems.
497-505	6	**Comments:** This is very dependent on the laboratory facilities available; nevertheless, the spectrum of potential investigations is large.
523	4	**Comments:** Modification for clarity. **Proposed change:**

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		…whether the results will be used to identify adverse effects due to ~~an extension of~~ ~~pharmacology,~~ **exaggerated pharmacologic effects, …**
530-534	5	**Comments:** In addition to cognitive and motor tests, behavioural battery testing should also include emotional and social tests, especially when pharmaceuticals are administered in periods that are critical for the development of these functions.
531	3	**Comments:** Concern is whether treatment of a ~~medicine~~ **pharmaceutical** with reproductive toxic potential…
539-542	4	**Comments:** The potential for confounding pharmacological effects can also apply to other neurobehavioral tests. Addition suggested. **Proposed change:** …should be considered and avoided, **as for other possibly affected assessments.**
549	4	**Comments:** Modification suggested since many CNS studies are conducted in mice. **Proposed change:** Postnatal CNS assessments are most commonly conducted and characterized in the ~~rat~~ **rodent**.
551-552	4	**Comments:** 'Learning and memory assessments are infrequently conducted in NHPs' is incorrect as learning tests are frequently conducted in ePPND studies (routinely in some labs). Learning tests are published and recommended for JAS in NHP older than 6 months (WGTA). Yet, there is no satisfactory memory test for NHP. **Proposed change:** Learning ~~and memory~~ assessments are infrequently…
560-563	4	**Comments:** Suggest removing "testicular immunohistochemistry" unless more specific detail is provided and why it is only relevant for male rodents. **Proposed change:** For concerns relevant for male rodents, sperm analysis (e.g., counts, motility, morphology) ~~and/or testicular~~ immunohistochemistry can be considered…"

Line no.	Stakeholder no.	Comment and rationale; proposed changes
565	3	**Comments:** What is the difference between a genetic sibling and a littermate – assuming that all littermates are genetic siblings.
571	3	**Comments:** being exposed to the ~~test~~ pharmaceutical
574-575	4	**Comments:** Add comma to improve clarity. **Proposed change:** In non-rodent species**,** mating assessments are not practical due to the protracted duration of development and high degree of individual variability.
582-585	4	**Comments:** Hormone assessments are variable at all ages, not just during puberty. If hormones are to be evaluated they should be powered appropriately. Modification to improve guidance and clarity. **Proposed change:** "…as there is considerable **variability in hormone measurements** ~~hormonal variability during puberty~~. Any hormone assessment should be justified, **powered appropriately,** and the timing and specific hormones assessed should be well characterized for the age **at which** the assessment ~~occurs~~ **is conducted**.
597	4	**Comments:** This section is too specific for a guidance document, appears prescriptive, is sometimes unclear, and there are contradictions. Suggestion to decrease level of detail and only leave 606-609 and 624-627. **Proposed change:** Delete several parts of section
599-627	6	**Comments:** JAS should be designed in such a way that all endpoints can be attained with minimal waste, maximum efficiency, and with scientific rigor for optimal results. In the case of preweaning allocation, the offspring are the test system and hence, the maternal animal should be provided with appropriate nutrition and care. A large litter size will affect the growth rate of each of the offspring with a smaller litter allowing for increased growth of the offspring due to better allocation of resources such as maternal care and food.
611-613	2	**Comments:** We suggest slight modification for clarity.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change: *"The repeat-dose toxicity studies to support FIH in adults could be performed in several ways;* ***standard repeat dose toxicity studies in two*** ~~*in both*~~ *species* ***using*** ~~*in*~~ *adult animals or* ~~*in*~~ *one or both* ~~*species*~~ ***studies could be conducted*** ~~*by initiating dosing*~~ *in juvenile animals* ~~*and continuing treatment*~~ ***with treatment continued*** *into maturity including additional endpoints."*
611-613	3	**Comments:** Language can be improved.
629-633	4	**Comments:** For postweaning allocation, the litter approach described in preweaning allocation is still recommended. However, a more efficient allocation can be 1/sex/litter/group since pups would no longer have covariates such as maternal care and littermates, and this approach would actually balance genetics/litter history across groups and also use less animals. **Proposed change:** revise section, give necessary flexibility
630-631	2	**Comments:** Can clarity be provided on when the nonclinical assessments of reproductive toxicity and carcinogenic potential would be warranted? Further clarifications would also be necessary on if and/or when these studies should be initiated in juvenile animals when it is determined they are required. **Proposed change:** We would welcome the mention of the criteria that should be considered when determining the need for these studies: treatment duration, age of paediatric population, treatment extends into adulthood, etc.
635	4	**Comments:** improved wording proposed **Proposed change:** …adequate number of animals to ~~evaluate~~ **do a meaningful evaluation of** the selected endpoints…
637-638	4	**Comments:** The option to use a single clinically relevant gender should be exploited. Addition to improve guidance. **Proposed change:** It is recommended that JAS be performed in both female and male animals,

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		unless the pharmaceutical is developed for one gender only.
Section 4	1	"In these cases, the FIH trial will be in paediatric patients and the nonclinical program, would generally include one JAS in a rodent and one JAS in a non-rodent species, if feasible. Safety pharmacology and genotoxicity testing would be conducted as appropriate for adults use; in vivo studies need not be conducted in juvenile animals". **Comments:** We do not support the current recommendation that the default approach for testing paediatric-first drug is to conduct two JAS in a rodent and a non-rodent species. According to a recent industry review on nonclinical safety considerations for the development of paediatric-first drugs, "consideration should given to conducting toxicity studies in adult rodent and nonrodent, followed by a juvenile study in the rodent only, provided this covers all concerns" and that only in certain occasions "where studies in adult animals are inappropriate for the clinical plan (e.g. in some rare disease indications)" would JAS in two species be warranted (Schmitt et al., 2016). In accordance with the 3Rs, it would be more appropriate to recommend, conditionally, the conduct of a single JAS and limit the conduct of additional JAS to rare cases only. **Proposed change:** In these cases, the FIH trial will be in paediatric patients and the nonclinical program, **may** ~~would generally~~ include one JAS ~~in a rodent and one JAS in a non-rodent species,~~ if **the weight of evidence raises safety concerns that cannot adequately be addressed in other nonclinical studies** ~~feasible~~. **Only in rare circumstances, (e.g. in some rare disease indications) might a second JAS also be considered.** Safety pharmacology and genotoxicity testing would be conducted as appropriate for adult use; in vivo studies need not be conducted in juvenile animals.
645-648	4	**Comments:** Addition to improve clarity. **Proposed change:** The repeat-dose toxicity studies to support FIH in adults could be performed in several ways; in both species in adult animals or in one or both species by initiating dosing in juvenile animals and continuing treatment into maturity including additional **relevant** endpoints (see Sections 2 and 3).
653-638	6	**Comments:** The number of animals used should be based on the desired endpoints. Excessive numbers should be avoided in order to conserve resources and limit waste. In general, larger numbers can result in behavioral factors related to

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		overcrowding, food distribution, etc. which may alter expected endpoints.
666-667	4	**Comments:** As for adult testing, the use of homologous proteins or relevant genetically modified animas can be appropriate for hazard detection and understanding the potential for adverse effects due to exaggerated pharmacology. Addition suggested. **Proposed change:** For biopharmaceuticals, studies in juvenile animals should be limited to relevant species, as per ICH S6. **The use of transgenic animals or homologous proteins should be considered when no relevant species exist.**
670	3	**Comments:** Is one dose for the extra group (combination treatment) sufficient? Which dose is recommended?
670	4	**Comments:** The current text leaves a gap for the potential use/need of NHP <10 months old at initiation of dosing in cases other than to support the use of perinatal and preweaning NHP for medicines with first and primarily neonatal clinical use (L674-676). Also, Table A1 states 'it is rarely feasible to initiate studies in juvenile monkeys <9 months of age', and NHP JAS starting younger have been performed (e.g., 6-7 months old NHP with burosumab to support ≥1 year old paediatric patients). Alignment for the lowest generally recommended age in a NHP JAS would be highly desirable (10 months?).
727	4	**Comments:** Note 1 is unclear and not guiding. reword or delete
731-732	4	**Comments:** Note 2 is too general and can discourage from useful procedures such as clinical observations. **Proposed change:** Study-related **invasive or prolonged** procedures should be limited as much as possible …
735	3	**Comments:** Add S8 – used as reference in line 551.
736-738	4	**Comments:** Note 3, particularly the last 2 phrases are unclear.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change: **For** JAS animals ~~are~~ generally ~~not screened~~ **no pre-dose data are generated** prior to initiation of treatment. Therefore, background rates of abnormalities (**e.g., eye findings**) in juveniles can differ from animals of the same age used in adult toxicity studies.
743	4	**Comments:** The note 5 would be easier to understand by making the difference between "absolute" and "relative" organ weights. Considering relative organ weights vs brain weight rather than BW could also be added.
747	4	**Comments:** add reference to ICH S8
Appendix A	4	**Comments:** Is there a reason why mouse and rabbit are not included in Appendix A, yet they are included in Table A1. **Proposed change:** consider to add mouse and rabbit.
Fig A.1	4	**Comments:** Some of the definitions are too dogmatic/strict (e.g., not all humans start solid food by age of 6 months, not all toddlers get breast fed until 2 years of age, puberty is not defined by age but Tanner stage >1 and can start in girls much earlier than by 11 years of age, pulmonary development is significant in first 2 years of age in human). **Proposed change:** update with help of (clinical) specialist
Figure A.1-A.5	4	**Comments:** No context is provided if the patient population was in the light hashed sections/age. **Proposed change:** Set expectations, specifically in Section 2 on the need for a study if the patient population impacted is 'lightly hashed'.
Appendix A	2	**Comments:** There are occasions when the mouse is the most relevant rodent species and may be used in JAS **Proposed change:** Age-dependent development for mice could be added (or clarify rat table can

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		be applied to mouse?)
Figure A.2 and Figure A.6	4	**Comments:** Conflicting information. Figure A.2 indicates a rat is adult at ca. PND70 vs. Figure A.6 indicates a rat is adult at ca. 9 weeks of age. Figures A.2 (but also A.1,3,4,5) indicate the immune system continues development until adulthood vs. Figure A.6 indicates development of the immune system ends in early adolescence (i.e., clearly before adulthood). In Figure A.6, the graded blue shading on the bars is unclear and some of the definitions are too dogmatic/strict (e.g., Human GI and Lung). And why is Fig. A.6 restricted to compare human to rat (why not other species)? Proposed change: Delete Figure A.6 (Figures A1 and A2-5 are more readily compared to provide the same information) or update with help of (clinical) specialist and align information between figures.
796 Table A1.	1	**Comments/Proposed change:** "Ethical reservations" are listed as one of the disadvantages to using NHPs in JAS in this table. According to a review on the need for juvenile animal studies, "in general, the use of animals for toxicity testing and in particular of young animals is a very emotional and controversial issue in our society and testing in monkeys and dogs is even less accepted than testing in rodents" (Soellner & Oleniczak, 2013). Another review stated that "animal use (especially in puppies or young monkeys) with no clear goal for risk assessment is totally unacceptable" (Baldrick, 2010). We therefore feel that it would be appropriate to also include "ethical reservations" as a disadvantage to using all species listed in JAS.
Appendix C	3	**Comments:** Appendix C Examples A and B: The examples are similar. Could you give an example for a different approach, e.g. cross-foster littering if considered appropriate?
815-824	4	**Comments:** For Case C, what if the CNS target was sufficiently well characterized to predict effects on developing CNS based on existing data (e.g., 3rd product with same pharmacology and patient population, and JAS with similar outcomes)? Issue with 'right to reference' (in USA) may exist. Such notion could also be added in Section 2. Addition/clarification suggested.

Line no.	Stakeholder no.	Comment and rationale; proposed changes
		Proposed change: …and expanded neuropathological examinations. **No JAS can be warranted if data from previous products in the same pharmacological class have adequately characterised the risk.**
831	4	**Comments:** clerical error. **Proposed change:** …decreased pharmaceutical Ig levels ~~was~~ **were** detected on PND 28 …
837 ff.	4	**Comments:** Overly detailed and corrections needed in App. C. For example, with a mean litter size of 11, only 45% of litters will have 5 male and 5 female pups. The majority of litters will have to be fostered, not “a very small percentage of pups” as stated. To avoid a possible bias of the mother towards its own pups, all pups must be cross-fostered so that no pup is raised by its biological mother. **Proposed change:** reduce level of detail

References from Stakeholder 1

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