European Medicines Agency

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OVERVIEW OF COMMENTS RECEIVED ON DRAFT GUIDELINE ON THE CLINICAL INVESTIGATION OF PHARMACOKINETICS OF THERAPEUTIC PROTEINS

Table 1: Organisations that commented on the draft Guideline as released for consultation

	Name of Organisation or individual	Country
1	EFPIA	
2	The International Plasma Fractionation Association (IPFA)	The Netherlands
3	Allergopharma	

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 86 13
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

GENERAL COMMENTS	Outcome
COMMENTS FROM EFPIA	
EFPIA commend the agency for a well-written guidance document on the clinical pharmacokinetic assessments for therapeutic proteins.	
The current draft guidance provides several useful recommendations, including a clear distinction for relevant studies for therapeutic proteins as opposed to small molecules. In general, the draft guideline allows consideration of testing on a case-by-case basis, as would be warranted given the diversity of structure and activity of therapeutic proteins, and limitations of current technologies.	
The guideline is vague in setting acceptance ranges for comparative bioequivalence studies, e.g. in the case of comparability exercises.	
COMMENTS FROM IPFA	
Generally, a very good guideline, well written More in the spirit of being advisory than practical This Guideline should be read in accordance and in harmony with all the specific therapeutic protein NfG already published by CPMP/CHMP.	
COMMENTS FROM ALLERGOPHARMA	
The nature of the therapeutic protein products that the Guideline is intended to cover should be clearly stated so as to avoid unnecessary discussion concerning the relevance to particular products. Most aspects covered are clearly directed at pharmacologically active proteins and peptides and are not appropriate for allergen products and in particular therapeutic allergen vaccines. These view is endorsed by the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95 – Section G)) which refers to the need to consider specific studies on a case by case basis, but states that pharmacokinetic studies are normally not needed. There is currently no specific guidance for therapeutic vaccines.	Partly accepted. In the introductory paragraph in section 3, Pharmacokinetic studies, it is stated that the pharmacokinetic requirements differ depending on the type of protein. To further emphasise the need for case-by-case considerations, the following has been added: *and intended use [of the protein]*.

1. INTRODUCTION		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
COMMENTS FROM EFPIA		
	There are several instances of abbreviations that are not explained. We suggest a glossary may be useful. Likewise, we suggest that references are provided for important examples	Partly accepted. Abbreviations have been explained in the text. It is not agreed to include references. This is not common practice for guidelines, except references to other guidelines.
Paragraph 2 p. 3/10 Sentence 1	*'One of the main objectives of the pharmacokinetic documentation is to ensure efficacy and safety in all patients, including sub-populations not represented in the Phase III trials'* The statement as it is sets unrealistic/unattainable expectations for pharmacokinetic analysis, which typically provides *supportive* safety and efficacy information. EFPIA suggests that the statement is modified as indicated: *'One of the main objectives of the pharmacokinetic documentation is to* contribute to assurance *of* ~~*ensure*~~ *efficacy and safety in all patients, including sub-populations not represented in the Phase III trials'*	Accepted
COMMENTS FROM IPFA		
P3, First paragraph	This guidance document should be read in conjunction with Directive 2001/83/EC, as amended, and all other pertinent elements outlined in current and future EU and ICH guidelines and regulations especially those on: o … o CPMP/BWP/1089/00 o CPMP/BWPG/283/00 o CPMP/BPWG/198/95 o CPMP/BPWG/388/95 o CPMP/BPWG/575/99 o CPMP/BPWG/ … And Note for Guidance on small populations	Not accepted. Describing pharmacokinetic requirements is not the main scope of the BWPG guidelines, and for other parts of the development plan it must be assumed that the sponsor reads the relevant guidelines within the specific therapeutic area. Also the Guideline on small populations concerns mainly efficacy and safety studies, and not pharmacokinetics.

1. INTRODUCTION		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
	Comment: Add all the existing BPWG Notes for guidance on the clinical investigation of plasma-derived Products	
P3, 1. Second paragraph	It is the objective of this document to: careful consideration during drug development ~~draw attention to dissimilarities in pharmacokinetic characteristics between proteins and conventional molecules affecting the content of the development program~~ Comment: Unnecessary: we know we are talking about Proteins	No change

2. BIOANALYSIS		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
COMMENTS FROM EFPIA		
2. Paragraph 1 page 3/10	It is stated in this section *"The ability to distinguish the therapeutically applied protein from endogenously produced equivalents should be considered in selecting the analytical method."* While the sponsors generally consider this factor, it is not always technically feasible to accomplish this task. It would be helpful to acknowledge this possibility in the guidance. Please add the following statement: *"It is recognized that developing an assay that distinguishes between the therapeutic agent and the endogenous molecule may not be always technically feasible."*	Accepted
2. page 3/10	Several "possible weaknesses" of bioanalytical methods which may result in erroneous characterisation of drug disposition, are listed for consideration by the applicant. The following issue could be added: Biological samples are often diluted prior to analysis by an immunoassay or a bioassay and the dilutions may cover a very large range. Dilutions between 1000- and 100 000-fold are often used. In such a situation, the linearity or parallelism of the applied dilution process should be tested and validated, both for quality control samples and for clinical samples. It should be demonstrated that results are independent of the applied sample dilution, as long as the measured concentration is within the validated calibration range.	Partly accepted. Dilution integrity is part of the normal validation of an assay and has been added to the list of aspects that should be studied during pre-study validation.
2.1 General considerations	Please clarify what is meant by 'bioassays which measure the activity of a compound **in a specific process'.** Is the author referring to bioassays that measure	Changed to "in a specific <u>biological</u> process".

2. BIOANALYSIS		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
Paragraph 1, page 4/10	the activity of a compound in a specific **biological pathway**?	
2.1 General considerations Paragraph 1, page 4/10	*'...a combination of immunological and bioassays is recommended for the clinical development.'* It is suggested that the section be re-worded to reflect the primary use of immunoassay methods to quantify the therapeutic protein in pharmacokinetic studies, with supplemental use of bioassays when needed and possible (due to methodological constraints). This would more accurately reflect current practices and standards. In practice, PK studies are seldom conducted with both a bioassay and an immunoassay. Immunoassays are the predominant tool used to quantify therapeutic proteins, with some emerging use of LC/MS/MS, (Liquid Chromatography/Mass Spectrometry/Mass Spectrometry), with peptides. As written, this section implies that both a drug quantitation method and a bioassay are required, which may not add data of value commensurate with the effort required. Suggested rewording of the section is as follows: 'While immunoassays are the predominant tool used to quantify therapeutic proteins, other assay methodologies such as LC/MS/MS or bioassays may be employed as well. A combination of assay methods may be appropriate if it is unclear if the assay employed is capable of measuring active test article.' Alternatively we suggest: *Due to the different characteristics of the methods and the entities being detected and quantified, a combination of immunological assays and bioassays is recommended for the clinical development <u>or the applicant should provide scientific rationale for exclusive or predominant use of either an immunological or bioassay approach</u>*	Partly accepted. The text has been re-arranged and a sentence has been added to make clear that exclusive or predominant use of one assay can be accepted if the applicant can provide a scientific rationale.
2.1 General considerations Paragraph 1, page 4/10	Methods should be adequately validated pre-study and within-study according to standard practice. In certain situations, wider acceptance criteria may be justified for therapeutic proteins compared with small molecules.	Agree. Acceptance criteria are not specified in this document. No change.
2.1 General considerations	5) lower limit of quantification **(LLOQ)** and **upper limit of quantification (ULOQ)** and limit of detection:	Accepted

2. BIOANALYSIS		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
Paragraph 2, page 4/10	*Comment:* The calibration functions are often s-shaped. Therefore, an ULOQ should also be validated. (6) ~~dose response relationship is determined~~: *Comment:* The expression should be substituted by: **dilutional linearity** (Also in this case: As calibration functions are often linear only within a small intervall due to the s-shape concentration response curve, high concentrations are to be diluted to the linear range. The linearity of dilution(s) is to be validated.	Accepted. Dilution linearity has been added.
2.1 General considerations Paragraph 2, page 4/10	Replace dose-response relationship with concentration-response relationship which is more correct, since in the development of an assay different concentration ranges of the analyte are tested.	Accepted
2.1 General considerations	As stated in 2.2 the Bioassay is not a reliable method to approach the PK profile of therapeutic proteins, accordingly EFPIA thinks there is too much weight on this assay in the general considerations of §2. EFPIA clearly does not agree with the sentence: *"Due to the different characteristics of the methods and the entities being detected and quantified, a combination of* *immunological assays and bioassays is recommended for the clinical development"* EFPIA might accept that the bioassay is a potential useful tool to validate the bioactivity of the immuno-reactive protein as detected in the ELISA. Bioassay is an inescapable test to control drug substance and drug product bioactivity but could not – in general - be used as a routine testing in PK studies.	See above.
2.2 Methodological problems Immunoassay 4/10	Under immunoassay: It may be important to also acknowledge an additional potential problem, relating specifically to monoclonal antibodies in this section (relevant to statements regarding bound and unbound fractions in Section 3.2, paragraph 4). That is, when the antigen binding site is required for capture and/or detection of the therapeutic monoclonal antibody, i.e., in a sandwich ELISA, it is important to recognize that one is only detecting free drug. This may result in PK characteristics that are biased or inaccurate, especially if free drug is a small fraction of the circulating material, and bound drug is important for efficacy.	Accepted. Binding to plasma components has been added under point (iv).
2.3 Endogenous concentrations page 5/10	*Knowledge of the concentration time profile of the endogenous component will facilitate the understanding of the exogenous component...'* Practical difficulties should also be acknowledged. Exogenous administration may	Comment acknowledged but detailed guidance on how to study endogenous concentrations is not considered to be within the scope of

2. BIOANALYSIS		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
	suppress production of the endogenous protein/polypeptide – in which case the pre-treatment endogenous profile may be of little practical value unless a specific endogenous marker, e.g., C-peptide for insulin, can be monitored. The following points might be added to enhance this section: "*A baseline assessment (≥ 24 hours prior to treatment) or a placebo arm can often be studied to differentiate pharmacokinetics of the exogenous and endogenous analytes. In the presence of a pulsatile baseline or diurnal variation, noncompartmental analyses are not appropriate. Modeling of the endogenous and exogenous concentrations early in development may allow one to design later studies and predict therapeutically relevant dosing regimens."*	this guideline.
COMMENTS FROM IPFA		
P4, 2.1 General Consideration, first paragraph	**2.1 General considerations** The most frequently used analytical methods for assaying therapeutic proteins in biological samples are i) ~~immunoassays~~, methods which estimate the amount of test compound that binds to a target antibody, e.e. immunoassays and ii) bioassays, which measure the activity of the compound in a specific process. Due to the different characteristics of the methods and the entities being detected and quantified, a combination of immunological assays and bioassays is recommended for the clinical development. Indeed, whereas immunoassays are able to detect structurally-related compounds, active or not, bioassays detect only active compounds, be they the parent product or its metabolites or any other structurally-related compounds, including endogenous proteins (see below). Other methodologies, such as LC-MS, may be used but are not specifically addressed here. If possible, it is preferable to develop a specific assay early in the development and use the same assay(s) during the entire development program. The difficulty of developing such an assay at an early stage is, however, recognised. Methods should be adequately validated pre-study and within-study according to standard practice. Difficulties may arise in the bio-analysis due to e.g. lack of specificity and some aspects important for the pharmacokinetic evaluation are highlighted in the section below. Comments:	

2. BIOANALYSIS		
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	- Detection assays might be of a different nature than immunoassays - LC-MS (?) Not in the abbreviation list - What is meant by Standard?	Potential use of other assays is mentioned later in the paragraph. Abbreviations has been explained References to ICH guidelines have been added.
P4, 2.1 General Consideration, 2nd paragraph	Validation of the analytical assay should comprise two distinct phases, (i) the pre-study phase in which the compliance of the assay with respect to (1) stability of the analyte in relevant matrix, (2) specificity, (3) accuracy, (4) precision, (5) limit of quantification and limit of detection, (6) dose-response relationship is determined and (ii) the within-study phase in which the method is applied to samples from a bio-study and control samples (QC and calibration standards) are used to confirm the correct performance of the run. **Comment:** Please, add references to texts on validation of analytical methods	References to ICH guidelines have been added.
P4, 2.2 Methodological problems Anti-drug antibody assay: (v)	(v) presence of the active substance may affect the ability to detect the anti-drug antibody since the antibody "is captured" by the active substance. Thus, when quantifying the anti-drug antibody the active substance should preferably be eliminated from the circulation Comment: Then, the assay would be irrelevant !	This paragraph concerns measuring antibodies towards the therapeutic protein. No change.
P5, 2.2 Bioassay (iii)	*(iii) presence of plasma components, e.g. binding proteins, inhibitors, drug antibodies may alter the activity of the analyte <u>or may reduce the efficacy of the active substance</u>*	The term activity is considered relevant when referring to the action of a drug in an *in vitro* assay, while the term efficacy is considered to be more relevant in the *in vivo* situation, which is not described here. No change.
P5, 2.2 Reference material, 2nd paragraph	*Contrary to conventional molecules, a pure reference material that can serve as a calibration standard is either difficult or sometimes impossible to obtain for this class of compounds.* *<u>Comment:</u> <u>It might be still necessary to define an internal reference/standard</u>. Therefore extreme care should be taken in order to ensure that the reference*	No change.

2. BIOANALYSIS		
Line no + paragraph no.	**Comment and Rationale**	**Outcome**
	material used in the different analytical calibration processes is representative of the material used in clinical trials, including clinical pharmacokinetics.	

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
3.1 second para	Investigating the extent of absorption would require intravenous studies in humans for drugs which are not intended for iv administration. This - in turn - will require an iv tox package. Such information may be useful for specific drugs, however is not considered essential in general. Determination of relative bioavailability at different administration sites in general does not seem feasible. The guidance states that relative bioavailability with respect to each administration site should be determined in clinical studies. While it is true that administration site can affect absorption characteristics, it is worth noting that when the dose of a therapeutic protein is large enough, the volume required for a single administration may necessitate multiple injections at multiple sites, and it may therefore be impractical and/or irrelevant to study the effect of different sites of administration. The optimal depth of an IM injection may be critical and vary across genders; thus an investigation of injection site effect needs to be balanced for gender. EFPIA propose that the first paragraph on page 6/10 is revised to state: *".....sites e.g. thigh abdomen and relative bioavailability with respect to each administration site should ~~be determined in~~ be considered regarding the need for clinical investigations~~studies~~. Other factors that might be considered in relation to their effect on the bioavailability are depth of the injection, concentration of the solution for injection, ~~and~~ volume of the injection and patient specific factors. "*	Partly accepted, EFPIA suggestion slightly reworded.
3.1 + paragraph 3 page 6/10	It appears that a full stop needs to be added in the middle of this sentence after the word efficacy.	Accepted

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
	Proposed rewording "*Sometimes, physico-chemical and in vitro biological analyses of the original and the modified version are not sufficient to exclude an impact on safety and efficacy. Thorough information about the…"*	
3.2 disposition	This section may be open to ambiguity in interpretation. It states that for therapeutic proteins, elimination is largely predictable based on the molecule size, and that the ultimate fate is catabolism. We suggest clarification that studies of the route of elimination and metabolism (including microsomal studies) should be considered on a case by case basis and are not generally required as the ultimate fate is proteolysis and reincorporation of the resulting amino acids in the physiological pool.	Accepted, see also comments on these paragraphs below
3.2 disposition Paragraph 1 page 6/10	For clarity, the first sentence "The main elimination pathway should be identified, which for therapeutic proteins could be predicted, to a larger extent, from the molecular size" should be modified as recommended here, however in addition please also taken the below comments into considerations EFPIA suggest rewording this section taking these comments into considerations, and also the importance of molecular size: "In general, the main elimination pathways of drug products should be identified. However, for therapeutic proteins this could be predicted, to large extent, from the molecular size."	Accepted
3.2 disposition Paragraph 1 page 6/10	The section starts with a summary of possible elimination pathways of therapeutic proteins. It is stated that "for larger protein molecules, **hepatic elimination** through receptor mediated endocytosis followed by catabolism is more important **relative to renal filtration**". It is certainly correct that elimination through endocytosis followed by catabolism is more important than renal filtration. However, is it really **hepatic elimination**? In a recent review article on antibody pharmacokinetics and pharmacodynamics (Lobo ED, Hansen RJ, Balthasar JP, J Pharm Sci 93: 2645-2668, 2004), the catabolism of proteins is discussed in the context of antibody distribution and clearance. It is stated that "antibodies are catabolized in tissues throughout the body" and that "the exact anatomical locations of antibody catabolism have not been identified". In the light of observations on the role of the Brambell receptor, FcRn, it is suggested that "endothelial cells may be the key cells involved in IgG catabolism". Thus, specifically for IgG proteins, hepatic elimination seems not to be the major	Agree.

3. PHARMACOKINETIC STUDIES		
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	elimination pathway. For many large protein molecules, as exemplifed by the immunoglobulins and albumin, the major elimination route is fluid phase pinocytosis by the reticuloendothelial cell system throughout the body. The half-life of elimination varies, such as 1-3 days (IgE) to 3-4 weeks (IgG). Some proteins, such as IgG, are "rescued" from lysosomal catabolism by specific receptors (FcRn discovered by Brambell). The statement regarding hepatic elimination is therefore not always correct, although some proteins, such as IgA, are taken up by receptor mediated endocytosis in the liver and transported to bile.	
3.2 disposition Paragraph 2 page 6/10	We commend the agency for clearly stating the issues related to conducting mass-balance studies with protein therapeutics and fully agree with this statement.	
3.2 disposition Paragraph 3 page 6/10	Polypeptides and smaller size proteins may be cleaved by proteases and amidases, which are typically cytosolic enzymes; hence microsomal preparations would not be the appropriate matrix to assess the metabolic pathways of such molecules. As the aforementioned enzymes are ubiquitous, they may be found in other tissue types as well. Therefore, it would be appropriate to use tissue homogenates as the first matrix and use whole cell preparations as the second matrix for such assessments rather than microsomal preparation. It would be possible to conduct metabolic studies in the above-mentioned matrices for polypeptides. However, generation of useful and interpretable data as well as the conduct of such studies could entail technical challenges for larger proteins. A brief reference to the potential limitations and issues involved with such studies would be useful.	Partly accepted. We suggest to replace this paragraph with the following sentence, which is inserted after the paragraph on mass-balance studies: *The need for, and the feasibility of, specific studies of the route of elimination and metabolism (e.g. microsomal, whole cell or tissue homogenate studies) and identification of metabolites in vitro should be considered and discussed on a case-by-case basis.*
3.2 disposition Paragraph 3 page 6/10	It should be clarified which classes of proteins do not metabolise conventionally (i.e. degradation to petides and amino acids) - an example would be helpful to clarify, because the Guidance S6 "Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals)" contains the passage in the text: "*The expected consequences of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids. Therefore, the metabolic pathways are generally understood. Classical biotransformation* studies as performed for pharmaceuticals are not needed." (page 8, chapter 3).	See comment above
3.2 disposition Paragraph 3 page 6/10	The guideline requests that "*the potential for hepatic microsomal metabolism should be considered especially for smaller proteins".* Proteins are normally catabolised by proteases in the lysosome. It is assumed that microsomal	See comment above

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
	metabolism means metabolism via CYP450 isozymes, similar to metablism of small molecules. Unless a protein or peptide can diffuse across a lipid bilayer, this is unlikely. Few proteins or peptides have been demonstrated to do this due to their hydrophilic physicochemical properties. Exceptions are likely to be small hydrophobic peptides. For these reasons it does not seem appropriate to recommend this approach.	
3.2 disposition Paragraph 3 page 6/10	With respect to metabolite issues, we appreciate the flexibility to consider doing metabolism studies only with small proteins and those for which metabolism may be different from normal proteins. However, it should be recognized that even in such cases, proteins may be metabolized to a large number of products and the identification of discreet structures for any given metabolite may not be possible with currently available technologies. The first statement of paragraph 4 of this section "Metabolites that have pharmacodynamic activity should preferably be measured, e.g. through chromatographic separation, collection and further in vivo bioassay quantification" is problematic and impractical since it would effectively require resource-intensive studies to isolate, purify and synthesize every metabolite for structure and activity characterization. Instead, we propose that the sentence be reworded as follow: "*Metabolites anticipated to have pharmacodynamic activity should preferably be measured when technically feasible.*	Partly accepted. The term "preferably" already gives the opportunity to do studies only when it is feasible. Instead we suggest adding a sentence on the possibility to measure the sum of active components, as suggested in the next comment.
3.2 disposition Paragraph 4 page 6/10	It is stated that metabolites that have pharmacodynamic activity should preferably be measured to characterise their pharmacokinetic properties, which may be different from that of the parent compound. This seems to be logical, but again may result in a big experimental issue. For instance, a small protein or oligopeptide (M) may be metabolised by subsequent loss of one amino acid, resulting in M-1, M-2, etc fragments. These fragments may still be pharmacologically active, but it may be very difficult to measure them separately. In this case an immunoassay which estimates the amount of a test compound that binds to a target antibody would give a "sum" signal and the PK based on this signal reflects the "sum" of the pharmakokinetics of the parent compound and the active fragments.	Accepted
3.2 disposition Paragraph 4 page 6/10	*'For some protein drugs, the activity is not only related to the unbound fraction in plasma but also to bound fraction'.* A specific example (and reference) would add credence to this statement since it	The paragraph has been changed to reflect that the conventional approach to discuss in terms of free fraction or free concentration may

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	is a general pharmakokinetic principle that drug [small molecule, peptide or protein] bound to *plasma* protein is inactive. A specific example where the bound fraction *per se* is active or toxic will clarify the guidance	not be fully appropriate. Peptides and proteins may be activated by complexing with plasma components. Binding to a carrier protein might modulate the activity at the target (e.g. IGFBP-1 modulating the activity of IGF-1). The "free fraction" may not be a very fixed measure, since the equilibrium between e.g. binding to carrier and binding to target depends on the affinity constants. As the mechanisms for the interaction between protein and binding components vary it is not considered appropriate to give an example or a reference.
3.2 disposition Paragraph 4 page 6/10	Reference to activity of a protein therapeutic bound to other proteins has been made in this section. If feasible, it would be useful to include literature references discussing such cases.	See above
3.2.1 Dose-and time dependency Paragraph 2, Page 7/10	It is stated "*It is recommended that the pharmacokinetics is determined at several dose levels and at several occasions during long-term studies."* Determination of pharmacokinetics of breakdown products (metabolites) of a protein may not be feasible due to the absence of sensitive and specific assay methodologies, thus we recommend clarifying that the PK determination refers to the therapeutic agent. Furthermore, the addition of a clarification regarding the utility of population pharmacokinetics in long-term trials would be useful.	Not accepted. The measurement of metabolites is discussed in the preceding section. When possible, also metabolites should be measured at several dose levels and in long-term studies. Accepted. A sentence on population pharmacokinetic analysis has been added.
3.2.2 Binding to blood components Paragraph 1 p. 7/10	We suggest that *"therapeutic"* be added before "*protein"* to read as suggested in order to improve clarity. *"Soluble receptors, e.g. shed antigens, may bind to the* ***therapeutic*** *protein resulting in altered pharmacokinetics through changed clearance or volume".*	Accepted.

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
3.2.2 Binding to blood components Paragraph 1 p. 7/10	Binding to blood components states that *"the binding capacity to plasma proteins (albumin, alph-acid glycoprotein) should be studied when considered relevant".* "**When considered relevant"** is very vague. No example is given, and thus, it remains unclear when this should be investigated.	No change is considered necessary.
3.2.5 Sub-populations Page 7 & 8/10	The guidance specifically encourages study of the effect of renal impairment on molecules smaller than 50,000 D because of the likely influence of filtration on clearance. We believe that it should also be noted that conditions that lead to renal impairment may affect the target load, which in turn could impact the pharmacokinetic properties and the efficacy of the protein. As such, the guidance might also note that the need for a renal impairment study should be carefully weighed based on the underlying biology/physiology of the therapeutic target regardless of the size of the therapeutic protein.	Accepted
3.2.6 Interaction studies Paragraph 1 & 2 p. 8/10	**paragraph 1:** last sentence: Literature references to the CYP1A2 effects will be especially helpful here. **paragraph 2:** *'An example of the latter is methotrexate, significantly decreasing the clearance of coadministered antibodies.'* EPIA suggest addition of the following two references with regard to the identified text: *« Maini RN. et al. 1998. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose* weekly methotrexate in rheumatoid arthritis. Arth & Rheum. 41(9):1552-63. *Velagopudi RB et al, 2003. Effect of methotrexate (MTX) coadministration on the pharmacokinetics (PK) of adalimumab (HUMIRA^{tm}, Abbott) following a single intravenous (IV) injection. ACR abstract 258."*	It is not agreed to include literature references. This is not common practice for guidelines, except references to other guidelines.
3.3 PK/PD-relationship Paragraph 1 p. 8/10 Line 2	*'If possible, established surrogate markers for both efficacy and safety should be measured preferably in the same study'* Virtually anything is "*possible*" with sufficient resources. A true "surrogate" marker [a statistically significant and strong predictor of therapeutic or safety outcome] is not known for most disease states. Therefore, we suggest that the 2nd sentence be revised to read: '*If feasible, markers for both efficacy and safety should be measured, preferably*	Accepted

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
	in the same study.'	
3.3 PK/PD-relationship Paragraph 1 p. 8/10	When considering PK/PD relationships, due consideration should be given to the expression level and turnover of the target ligand to which the protein binds and changes in this which may occur with disease (including though not exclusively organ impairment). Most protein drugs are administered at doses which are low, in molar terms, compared with classical low molecular weight chemicals. Therefore concentrations of drug and those of the target may be similar, such that the kinetics of the drug become dependent upon the kinetics of the target. Under these conditions the assumptions implicit in the classical Langmuir or Hill equations, that the receptor is at low concentrations relative to the substrate, may not be valid. If this condition is met, PK/PD models should thus be formulated to describe the binding reaction(s) explicitly and measurements taken of the binding target, if feasible, perhaps by receptor assays.	Accepted.
3.3 PK/PD-relationship (re. line 8)	Specify "*in silico models*" in order to distinguish from in vivo models.	Partly accepted, the text has been changed to "PK/PD models".
3.4 Immunogenicity Paragraph 2 Page 9/10	As stated binding antibodies, can interfere with the pharmakokinetics of proteins, but they also can interfere with the detection method, this point is not addressed in the guideline and might be helpful for interpretation. *"Since anti-drug antibodies may alter the pharmacokinetics and pharmacodynamics of a protein, testing for antibody response is always necessary when developing a new protein. It is especially important for new drugs intended for multiple-dose or long-term treatment."* EFPIA would suggest to underline that binding anti-drug antibodies can either prolong the half life of a protein or on the opposite increase its clearance. Data interpretation should be carefully assessed, since these results might be altered by direct interference of these antibodies in the ELISA detection system, through competition with the antibodies used within this assay technology.	Partly accepted. Paragraph 3 in this section already mentions that CL might either increase or decrease due to antibodies. It is not considered necessary to further underline this. A sentence regarding risk for interference with analytical assay has been added to the 4th paragraph.
3.4 Immunogenicity Paragraph 2 Page 9/10	The guidance recommends that samples (for anti-drug antibodies) should be collected when the drug concentration is low enough not to interfere with the analysis, *i.e.*, after 6-7 half-lives and when anti-drug antibodies have developed. However, the time frame of 6-7 half-lives is not practical for products that are given chronically. The document should provide guidance for the minimum number (and timing) of samples that need to be collected to assess anti-drug	Text has been slightly revised but exact guidance cannot be provided, as this must be determined on a case-by-case basis.

3. PHARMACOKINETIC STUDIES		
Line no + paragraph no.	**Comment and Rationale**	**Proposed change (if applicable)**
	antibodies under situations of chronic use**.**	
3.4 Immunogenicity Paragraph 4, Page 9/10	It is implied that all patients from a phase III program should have PK samples withdrawn after the first and the last dose in order to evaluate effects of antibody formation. However, it should be specified that this may be studied in a fraction of the patients by means of a Population approach in which antibody presence is treated as a covariate.	Partly accepted. The number of patients needed to detect a signal of antibody formation may be very large, if the frequency of antibody formation is low, and by only sampling a fraction of patient the signal may be missed.
3.4 Immunogenicity Paragraph 4, Page 9/10	Correlating exposure to the onset and degree of antibody response, especially in patients who withdraw from late phase trials: While we agree with encouraging the use of population methodologies and feel that this may be a necessary first step in facilitating the type of correlation requested in the guidance, greater emphasis should be placed on establishment of accurate PK models early in development if samples drawn on withdrawal are to be used as surrogates for exposure. The guidance should acknowledge that obtaining a sample at some random time after a patient withdraws from a study is of dubious value unless one has a good understanding of exposure as a function of the concentration at any given time following a dose. In addition, it should also be acknowledged that even well-characterized exposure from one particular time period in a trial may not be reflective of the exposure experienced at the time of a withdrawal or adverse event. The bottom line is that prospective data analysis planning, founded in the body of knowledge about the disposition of the molecule together with the cumulative experience of antibody response to drug, is critical to allow meaningful exposure-response interpretation of AEs surrounding patient withdrawal.	The comment is acknowledged, but the suggested level of detail for model development is not considered relevant for this guideline.
3.4 Immunogenicity Paragraph 4, Page 9/10	It is recommended that this section is supplemented with the adjacent text. Proposed rewording *"The presence of anti-therapeutic protein antibodies should be determined using both an immunoassay for the presence of binding antibodies and a biological assay for the presence of neutralizing antibodies. It is important that the assays be fully validated, sufficiently sensitive to detect clinically relevant antibodies, be able to detect all classes of antibodies, and be able to detect the presence of rapidly dissociating (low affinity) antibodies."*	Accepted, with slightly shortened text, as "all classes of antibodies" may be considered to be covered by the text "clinically relevant antibodies".
3.5 Comparability	The guideline is too vague in setting acceptance ranges for comparative	Not accepted. Comparability is generally discussed in more detail

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	bioequivalence studies, e.g. in the case of comparability exercises. More specificity should be introduced into this section, e.g. by quoting examples for acceptable practice in setting the acceptance range.	in the different guidelines for specific therapeutic areas. It is not considered necessary or feasible to go into detail here.
	It is stated that demonstration of equivalence on clearance and/or half-life may be necessary in a clinical comparability trial. It would be more appropriate to use the word 'comparability' rather than equivalence for a protein therapeutic, considering that conventional bioequivalence study designs may not be feasible for most proteins as stated in the draft guidance. Considering that clearance is inversely proportional to area under the concentration-time curve, the assessment of PK comparability using AUC would not yield a result different from that using clearance. Additionally, half-life could be a very variable parameter and is affected by clearance as well. Thus, the requirement for establishing equivalence using half-life would not be appropriate. Proposed rewording: "I*n fact, the risk of differences in elimination rate may be more likely, requiring the demonstration of comparability on clearance".*	Accepted
	EFPIA thinks it is important to draw investigators'attention to a key pharmakokinetic parameter for such proteins which is the **Mean residence time (MRT)**. The draft appropriately states the importance of clearance and half life: *"Since not only similarity in terms of absorption/bioavailability is of interest, the standard bioequivalence design may not be optimal. In fact, the risk of differences in elimination rate may be more likely, requiring the demonstration of equivalence on clearance and/or half-life."* EFPIA would suggest to emphasize that if the Area under curve (AUC) is a critical parameter when comparing bioequivalence of two small molecule drugs, the experience shows that the **MRT** or the **average residence time over a certain blood level considered as threshold for activity**, is very critical to demonstrate comparability. This is linked to the rather high affinity of these proteins to their target and this explains why most Pegylated or heavily glycosylated proteins are given at doses lower than the cumulated dose of their naked protein counterpart over the same time period. It is interesting to note that if the IV route and its 100% absorption, apparently increases protein AUC, by increasing the clearance and decreasing the MRT, it can in fact lead to a	Not accepted. It may generally be more difficult to obtain a reliable estimate of MRT, and the current wording is in line with the Comparability guideline EMEA/CHMP/42832/2005.

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	decreased pharmacodynamic effect in comparison to the SC route.	
	The addition of a list of definitions and terms would be useful to the reader. Terms such as small size protein and large size protein could be defined in such a section.	A list of definitions is not considered necessary.
COMMENTS FROM IPFA		
P5, 3. PK Studies, 1st paragraph	*Generally, the requirements for therapeutic proteins with respect to evaluating the pharmacokinetics of the product are the same as for conventional products, but specific considerations are needed related to the inherent characteristics of proteins. The pharmacokinetics (absorption, distribution and elimination) should be characterised during single-dose and steady-state conditions in relevant populations. However, the pharmacokinetic requirements may differ depending on the type of protein.* Comment: Most of the PK studies are not in a steady state condition and Blood Product Working group (BPWG) guidelines (FVIII, FIX…) do not require such a condition. An harmonisation between the two guidelines might be necessary	It is agreed that harmonisation between guidelines is desirable. Some determination of pharmacokinetics at steady state – e.g. by sparse sampling in phase III studies – may be considered a minimum requirement. The text in the present guideline is therefore considered adequate for a guideline on peptides and proteins in general. No change.
P6, 3.1.absorption 2nd paragraph	*Changes in formulation or in the manufacturing process of the drug substance may alter the pharmacokinetics and the immunogenicity of a compound (Section 3.4). Sometimes, physico-chemical and in vitro biological analyses of the original and the modified version are not sufficient to exclude an impact on safety and efficacy. Thorough information about the pharmacokinetics and the relationship between concentrations and efficacy and safety (PK/PD) might in some situations reduce the need for clinical studies.* Comment: In the BPWG NfG, it is indicated, "*The effects of changes in the manufacturing process must be investigated. If significant impact on the activity of the therapeutic protein cannot be excluded, data on kinetics must be provided."* An harmonisation between the two guideline might be necessary	It is agreed that harmonisation between guidelines is desirable. Pharmacokinetic data alone may not always be sufficient to exclude effects of manufacturing changes on efficacy and safety of the protein. The text in the present guideline is therefore considered adequate. No change.
P6, 3.2.Disposition 4th paragraph	*For some protein drugs, the activity is not only related to the unbound fraction in plasma but also to the bound fraction.*	Accepted
P6, 3.2.Disposition 5th paragraph	*For larger proteins, Vss is similar to the distribution of albumin (approximately 0.1~~L~~ l /kg).* Comment: QRD: International units are in lower case: l/kg and not L/kg	Accepted
P7, 3.2.4	*The inter-subject variability should be estimated and if possible the important*	Agree. Therefore, the guideline

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Variability, First Paragraph	*sources of the variability identified e.g. demographic factors as weight and age.* Comment: Often not possible due to small sample sizes of PK studies (in rare diseases)	states sources of variability should be determined "if possible". No change.
P7, 3.2.5 Sub-population First Paragraph	*An understanding of the influence of intrinsic factors, such as age and body weight should be provided. Such information might arise from conventional studies in a specific population or from population pharmacokinetic analysis of Phase II/III data.* Comment: Most of the time, dose of products are given by units per body weight. The PK parameters include the "kg" in the results, integrated directly!	If dosing per bodyweight is suggested, this should in general be supported by data showing how body weight influences the pharmacokinetics. No change.
P9, 3rd paragraph	*Antibody formation can cause increased or decreased clearance (CL) of the therapeutic protein, although the former effect is the most common.*	Accepted.
P9, 3.5 Comparability	*Demonstration of comparability between two products is most often a step-wise procedure where pharmacokinetic data are an important part, when needed.* Comment: In the BPWG NfG, it is indicated, "The effects of changes in the manufacturing process must be investigated. If significant impact on the activity of the therapeutic protein cannot be excluded, data on kinetics must be provided." An harmonisation between the two guideline might be necessary	Not accepted. The guideline states "most often". Further down in the paragraph, it states that pharmacokinetic studies are "often" necessary. This is considered sufficient to underline that pharmacokinetic data may not always be the necessary to show comparability.
P10, 2nd paragraph	*The acceptance range to conclude equivalence with respect to any pharmacokinetic parameter should be based on a clinical judgement, taking all available efficacy and safety information on the reference and test products into consideration. Hence, the criteria used in standard bioequivalence studies may not be appropriate and the equivalence limits should be defined and justified prior to conducting the study.* Comment: It is not so easy to define *Clinical* bioequivalence limits. And usually the 80%-125% limits used for traditional PK studies are defined as the margin of bioequivalence. For plasmatic proteins, limits are never (to our knowledge) assessed clinically. However, if they exist, it is fine to agree to use them!	Comparability is generally discussed in more detail in the different guidelines for specific therapeutic areas. It is neither considered necessary nor feasible to go into detail here.