11 November 2013
EMA/682105/2012
Committee for Medicinal Products for Human use (CHMP)

Overview of comments received on “Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product”

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

Stakeholder no.	Name of organisation or individual
1	Commission on Human Medicines - UK
2	EGA
3	Ely Lilly
4	Hoffman-LaRoche
5	GILEAD
6	Hexal
7	IFAR
8	MEB - NL
9	Szebeni

7 Westferry Circus ● Canary Wharf ● London E14 4HB ● United Kingdom
Telephone +44 (0)20 7418 8400 **Facsimile** +44 (0)20 7418 8416
E-mail info@ema.europa.eu **Website** www.ema.europa.eu
An agency of the European Union

1. General comments

Stakeholder number	General comment (if any)	Outcome (if applicable)
1	1. The paper would benefit from a glossary of terms to help clarify terminology used to describe the association of the active substance to the liposome e.g. entrapped, unentrapped, associated, free (free from liposome or free from protein-binding?), encapsulated, unencapsulated, total. 2. Prior to finalisation of the reflection paper, consideration should be given to organising a Joint EMA-Industry meeting to discuss the technical feasibility of particular aspects relating to the non-clinical and clinical comparability studies (e.g. the capability of current analytical tools and methodology to conduct the proposed tissue distribution and PK studies).	Accepted A short Glossary is provided Members of the working parties welcome any opportunity to discuss the scientific basis of this or other guidance documents in scientific meetings and the importance of open scientific dialogue with the stakeholders is recognized. In this instance such dialogue may not be possible, as organising a separate meeting before the final release of this document may result in a considerable delay. However, a Joint EMA-Industry meeting should be considered based on the need of such a meeting and the available resources.
2	1. The EGA welcomes the EMA Reflection Paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product and the opportunity provided for industry to comment on the EMA considerations. 2. EGA member companies are in favour of EMA developing a guideline which would address the key general principles of intravenous liposomal formulation	Accepted EMA is actively seeking to develop scientific cooperation with the corresponding regulatory bodies of US, Canada and Japan. But the scope of this guideline is not considered an ICH topic and

Stakeholder number	General comment (if any)	Outcome (if applicable)
	development allowing applicants to scientifically justify their development strategy, seeking through Scientific Advice, where felt needed, the opinion of regulatory authorities. The EGA would encourage early and extended discussions between the EMA and the US FDA in order to achieve a common understanding of the general principles of liposomal product development. This would encourage the development of complex generic medicinal products. It would also prevent the unnecessary duplication of pharmaceutical development programmes, especially for this complicated category of formulation where a limited number of companies will be able to technically engage in such development.	harmonization is not expected in the near future.
3	Because the EMA has not issued any guidelines on data requirements for the development of liposomal products, this reflection paper covers considerable subject matter. It would be beneficial to: • focus one guideline on the development requirements of liposomal products, • and then focus a second guideline on the comparability requirements for liposomal products developed with reference to an innovator liposomal product.	This reflection paper already states: “The principles outlined in this reflection paper might also be considered to be applicable to other novel types of “liposome-like” and vesicular products which may be under development including those to be administered by routes other than intravenous administration”
4	We welcome this reflection paper and its intention to address and provide guidance to innovator companies to expedite the development of liposomal products for human use.	Accepted

Stakeholder number	General comment (if any)	Outcome (if applicable)
	It is very timely that a scientific and regulatory feedback is being provided with a renewed perspective. In principal, we agree with most of the points in the reflection paper and have added some specific additional comments for consideration (see below).	
5	Gilead thanks the agency for the opportunity to submit comments and suggestions on the Reflection paper. We find the paper well considered and informed, and find that it takes the consequences and manifestations of non-equivalence seriously.	Accepted
6	According to the EMA reflection paper, several preclinical PD and PK studies (and toxicity studies) in addition to BE studies with each recommended doses are required for a generic application for a liposomal formulation. However, the FDA published a product-specific draft guidance on liposomal doxorubicin HCl in 2010; according to this guidance, one BE study in patients with the highest dose is required for a generic approach. We propose to consider a harmonization of requirements for a generic approach for liposomal products between EMA and FDA.	EMA is actively seeking to develop scientific cooperation with the corresponding regulatory bodies of US, Canada and Japan. But the scope of this guideline is not considered an ICH topic and harmonization is not expected in the near future.
7	IFAPP is in agreement with the contents of the document	Comment noted
8	This draft reflection paper gives an adequate overview of the issues surrounding 'generic' liposomal products. Overall we agree with the proposals made in this paper	Partly accepted. It is agreed that developing product-specific guidelines is a proposal for future consideration. However, it is beyond the mandate and scope of this reflection paper.

Stakeholder number	General comment (if any)	Outcome (if applicable)
	regarding strategies for development of generic liposomal products. We fully agree to the statement that the aims of the original development of the liposomal formulation and the supporting evidence should be taken into account in the requested support for registration of such 'generic' liposomal products. On the other hand, when the innovator drug is not defined as extensively as present day requirements demand regarding quality, PK pharmacodynamic and clinical aspects, the Applicant of the generics may be forced to perform more elaborate analysed and comparative studies on quality and preclinical aspects than the company of the innovator drug. The proposal that, regarding the assessment of a new generic liposomal drugs for i.v. use, apart from general guidelines, specific and more 'customised' requirements as communicated in a scientific advice must be followed, is endorsed. It is agreed that Applicants developing such liposomal products should make utmost attempts to demonstrate equivalence based on pharmaceutical, non-clinical pharmacokinetic (as well as pharmacodynamic, see specific comments) and clinical pharmacokinetic studies, since comparative clinical efficacy trials may be less sensitive to detect formulation related differences. Some detailed comments for further improvement of	

Stakeholder number	General comment (if any)	Outcome (if applicable)
	the paper are listed below.	

2. Specific comments on text

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
Line 18-20	8	As "poorly water soluble" and "unstable" are not necessarily linked, NL recommends to amend the sentence as following: *highly lipophilic/poorly water soluble compounds, unstable compounds, or …* Please consider deleting "*highly water soluble*" at the end of the sentence as poorly water soluble drugs are not excluded.	Accepted. The sentence is reworded to be more general and less specific.
19-20	4	Comment: The aspect of tissue targeting should not be restricted to only water soluble compounds. Proposed change (if any): "[…], or for tissue targeting of **new molecular entities**. ~~highly water soluble compounds."~~	Accepted. The sentence is reworded to be more general and less specific.
21-24	5	Comment: The sentence following "Liposomes are…" includes structures that cannot fall into the given definition. Proposed change (if any): replace "Such" with "Outside of this definition there are variants…"	Accepted
Lines 21-24	8	NL proposes to complete the sentences as following in order to be more precise: *Liposomes are classically described as artificially prepared vesicles composed of one or more concentric lipidic bi-layers enclosing one or more aqueous compartments. They include, but are not limited to, mono- and multi-lamellar liposomes, multi-vesicular liposomes, polymer-coated liposomes and*	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		some drug-lipidic complexes. With regard to the latter, it is noted that not all lipidic complexes correspond to liposomes. Could the latter be deleted?	
Line 34	8	Please replace "*in vivo*" by "*after intravenous administration*" to keep in line with the title of the reflection paper.	Accepted
34-35	5	Comment: In addition to formulation, specific manufacturing and raw material and quality control attributes are often equally critical. References 1 and 2 provide examples of such cases. Proposed change (if any): Add a sentence after "performance" on line 35 that says: "Even for cases of ostensibly identical composition, variation in production and product and process control technology can lead to products with very different therapeutic performance".	Accepted
Lines 42-43	1	Comment: Current legal requirements necessitate that the innovator product used as a reference should be sourced from within the EU market. The lines indicated should be clarified to specify that this requirement applies to all pivotal non-clinical and clinical studies. Proposed change: The reference liposomal product used for comparability investigations should be sourced from within the EU and should be used as a comparator in all proposed quality characterisation and pivotal non-clinical and clinical	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		comparability studies.	
42-43	2	Comment: 'The reference liposomal product used for comparability investigations should be sourced from within the EU and should be used as a comparator in all proposed characterization studies.' This terminology is not fully in line with the one in the EU pharmaceutical legislation which reads: 'the medicinal product is a generic of a reference medicinal product which is or has been authorised under Article 6 for not less than eight years in a Member State or in the Community.' (article 10, paragraph 1, Dir. 2001/83/EC, as amended). Proposed change (if any): 'The reference liposomal product used for comparability investigations should be a reference medicinal product which is or has been authorised under Article 6 for not less than eight years in a Member State or in the Community.' (article 10, paragraph 1, Dir. 2001/83/EC, as amended) ~~sourced~~ from within the EU and should be used as a comparator in all proposed characterization studies.'	Accepted in a shortened form.
Lines 42-43	3	Comment: The term "sourced from within the EU" is unclear. Indeed, if the reference product marketed in the EU is manufactured elsewhere, it is our assumption that the product would be considered as an appropriate reference	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		product, however it is not clear. Proposed change (if any): "The reference liposomal product used for comparability investigations should be ~~sourced from within~~ or have been authorised in the EEA, and should be used [...]".	
Line 68-74	1	Comment: In view of the paper recommending non-clinical animal comparability studies, the following comment should be considered for insertion into a more prominent part of the paper e.g. into the 'Scope' as an additional bullet: Proposed change: Insertion of an additional bullet: Hence, this document should facilitate a decision on the following issues: • pharmaceutical data needed as evidence of product comparability between test and reference or after changes to a liposomal product, to support comparative safety and efficacy • Necessity of pre-clinical and clinical studies (including 'usual' bioequivalence studies) and circumstances which may allow to waive certain studies • Consideration of the design of relevant in vivo non-clinical studies with reference to the 3Rs and the potential role for in vitro models.	Accepted
77	5	Comment: After the inclusion of "Liposome-like" and vesicular products, a small section could be added to specifically exclude systems that may use phospholipids but	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		where drug release is unaffected; i.e., where the lipidic system serves only as a solvent for the active agent. An example of such a system is provided in Reference 3 Proposed change (if any): To add the aforementioned sentence.	
Lines 89-91	1	Comment: This statement should be clarified to specify that pharmaceutical comparability should be established first, and that such comparability does not substitute for (but rather complements) the establishment of comparability in the non-clinical and clinical studies described in later sections of the reflection paper. The extent and complexity of clinical and non-clinical studies should be driven by the results of the comparability work at each stage. Proposed change: Pharmaceutical comparability between the applicant's product and the innovator product should be established before progressing to non-clinical and clinical investigations. Due to the complexity of liposomal formulations, establishing pharmaceutical comparability alone cannot replace the need for ~~substitute entirely for~~ non-clinical and/or clinical data but may justify reduction in the amount of such ~~non-clinical and clinical~~ studies. The extent and complexity of clinical and non-clinical studies should be driven by the results of the comparability work at each stage.	Accepted. The proposed text has been added.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
Lines 89-91	6	Comment: *The text states: "Due to the complexity of liposomal formulations, establishing pharmaceutical comparability cannot substitute entirely for non-clinical and/or clinical data but may justify reduction in the amount of non-clinical and clinical studies*." We propose to specify which pre-conditions have to be met that the amount of non-clinical and clinical studies can be reduced and which kind of studies can be waived.	Accepted. The corresponding non-clinical and clinical sections are restructured and considerably rewritten. It is believed that these sections provide straightforward answers to the questions raised in the comment.
Lines 92-94	8	This section makes no reference to analytical methods. It would be helpful to add exemplary acceptable methods or refer to relevant literature.	Not accepted. It is agreed that reference to analytical methods would be helpful. Nevertheless, currently there is no complete common consent on the analytical methods to be used. Presenting a non-complete list could rather confuse and cause misunderstandings.
Lines 93-95	8	NL proposes to simplify the beginning of the sentence to: *Correctly ~~identifying the parameters that define~~ <u>defining</u> relevant physicochemical properties…* Aspects such as reproducibility and uniformity of the manufacturing process, validation of the manufacturing process, critical evaluation and validation of up-scaling, removal of process reagents, sterility (aseptic processing or sterile filtration) are lacking in the section on quality characterisation. Although it is acknowledged that this applies not to liposomal products only, NL recommends addition of these parameters here or in the section on pharmaceutical development in order to avoid	Comment noted. The words 'identifying the parameters that define' have been replace by 'defining' Since the reflection paper should primarily focus on attributes specific for liposomal formulation a general statement was added: "The liposomal product shall, with regard to quality data, fulfil all requirements for Module 3 as defined in Annex I to Directive 2001/83/EC and satisfy the technical requirements of the monographs of the

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		misunderstandings with Applicants.	European Pharmacopoeia and any additional requirements, such as defined in relevant CHMP and ICH guidelines."
Lines 96-97	8	The level of detail required for the information on lipidic components is currently not clear. For instance, the FDA expects the same level of detail as for a drug substance in their guidance on liposome drug products (section II D) while the draft reflection paper leaves this open. NL recommends to specify the expected level of detail in order to facilitate composition and assessment of MA dossiers.	Accepted The following text has been added: "The quality and purity of the lipid starting materials is essential for the later quality of the drug product, therefore the appropriate characterization and specification of the lipid starting material is considered as vital. Functionality-related characteristics as described in the Ph. Eur. monograph 5.15 'Functionality-related characteristics of excipients' should be adequately addressed. The level of information to be provided with the relevant submission depends on complexity of the excipients. The principles of the Guideline on Excipients in the Dossier for Application for Marketing Authorisation of a Medicinal Product (EMEA/CHMP/QWP/396951/2006) should be considered. Use of multiple sources (e. g. animal, plant, synthetic sources) or suppliers for the lipid components would require additional characterisation and comparability studies.
Lines 96-98	3	Comment: The inclusion of "stability" as an area to be	Comment noted.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		addressed may give the impression that there is an expectation of stability data for excipients. If so, there should be further scientific justification for this requirement. Proposed change (if any): Delete "stability" from line 96 and 98.	To avoid misunderstanding 'stability' has been replaced by 'stability characteristics'
Line 97	4	Comment: Some aspects should be added to this bullet point. Proposed change (if any): […] manufacture, specification, **purity/impurity profile, isomers,** and stability);	Accepted The words 'purity/impurity profile, isomers' have been added
Line 98	3	Comment: Further definition is needed for "nonlipidic starting materials" and how these differ from "critical excipients". Proposed change (if any): "quality, purity and stability of other ~~nonlipidic starting materials and~~ critical excipients"	Accepted The words 'nonlipidic starting materials and' have been deleted.
Lines 100-101	4	Proposed change: Active substance/lipid moiety ratio at relevant manufacturing steps **to be within acceptable range** to ensure consistent formulation **performance**;	Accepted. The words 'to be within acceptable range' and 'performance' have been added.
Line 102	3	Comment: The wording under this bullet point is unclear. Proposed change (if any): "liposome morphology, **mean** size and size distribution"	Accepted The word 'mean' has been added.
Line 102	8	Please amend the bullet point with aggregates: - liposome morphology, size and size distribution, aggregates	Comment noted. The word 'aggregates' has been added.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		It is noted that, in contrast to the FDA guidance, the volume of entrapment is not included in the list. NL recommends that inclusion of this parameters is considered.	Attributes like size, size distribution, lamellarity and fraction of encapsulated active substance are addressed in the reflection paper. Volume of entrapment will not give considerable additional information. Furthermore, there is no standard method available to measure volume of entrapment.
Line 104	8	Please amend the bullet point as following: - assay <u>of active ingredient</u> and lipidic components	Accepted. The words 'of active ingredient' have been added.
Line 105	4	Proposed change: ~~osmolarity;~~ **Osmolality of the delivery vehicle for information trending;**	Comment noted. Osmolality and pH are general requirements for parenteral formulations. Therefore they have been deleted. A general statement: Since the reflection paper should primarily focus on attributes specific for liposomal formulation a general statement was added: "The liposomal product shall, with regard to quality data, fulfil all requirements for Module 3 as defined in Annex I to Directive 2001/83/EC and satisfy the technical requirements of the monographs of the European Pharmacopoeia and any additional requirements, such as defined in relevant CHMP and ICH guidelines."
Line 105	8	Please amend the bullet point as following as pH may be	Comment noted.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		relevant for the stability of the drug substance and lipidic components: - osmolarity, pH	pH is a general requirement for parenteral formulations (see comment above). The words 'pH of internal compartment' have been added in another section of the text to be more specific.
After line 105	8	As these aspects are not covered yet, NL recommends to add the following bullet points: - sterility assurance - pyrogens/bacterial endotoxins - removal of process reagents	Not accepted. The bullet points addressed in the comment are also relevant for all other parenteral products. This RP should primarily address topics relevant for liposomes. A general statement has been added (see comments above).
Line 109	4	Proposed change: Stability studies under proposed in-use conditions **and a reference higher temperature for accelerated stability assessment;**	Not accepted. Assessment will be case-by-case decision.
Line 110	3	Comment: Agency guidance is needed on the term "stress conditions" as this term is not used consistently by health authorities, while there is a need for the sponsor to understand how drug release may be altered following in-use conditions. Proposed change (if any): "in *vitro* drug substance release rate from the liposome in relevant media and ~~stress~~ **simulated in-use** conditions"	Accepted. The word 'stress' has been replaced by 'simulated in-use'
Line 110	4	Proposed change:	Not accepted.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		In vitro drug substance release rate from the liposome in relevant media and stress conditions **using suitable method for assessing in-vitro release rate;**	Suitability of in-vitro assay is addressed in lines 129-134.
Line 110	6	Comment: *The text states: "in vitro drug substance release rate from the liposome in relevant media and stress conditions"* As the release of active substance under physiological conditions should in the most cases be avoided, we propose to clarify if an in vitro leakage test (e.g., described in the FDA Draft Guidance on Doxorubicin) in relevant media and stress conditions is more appropriate than showing a release profile.	Comment noted. The following words have been added: 'If justified an in-vitro leakage test in relevant media under multiple conditions (e.g. range of temperatures and pH values) could be appropriate.'
Line 110	8	Comment: Later in the document at lines 129-134, the type of in vitro release methods is specified. This could be combined with line 110. Proposed change: Combine line 110 with 129-134.	Not accepted. In-vitro release is crucial. Therefore after the list with the bullet point a separate paragraph dealing with in vitro release is included.
Line 111	3	Comment: The use of the term "validated" is not appropriate for this type of characterisation test. Proposed change (if any): "~~validated~~ process for reconstitution and/or pharmacy preparation"	Acceptable. The word 'validated' has been deleted. It has been replaced by 'Robustness of'
Line 116	4	Proposed change: Maintenance of liposomal formulation integrity in plasma **and target tissue of interest;**	Not Accepted. The proposed change is more relevant for non-clinical studies and is addressed in the non-clinical and clinical sections of the reflection paper.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
Line 117	2	Comment: The text is ambiguous and the punctuation seems missing (coma). Proposed change (if any): In line 117, please reword as 'characterisation/specification testing for lipid bilayer phase**,** transition temperature and/or liposomal 'surface' charge'.	Comment noted. The text has been reworded to: • characterisation of lipid bilayer phase transition behaviour (e.g. temperature and enthalpy of transitions)
Line 117	3	Comment: The guideline recommends that lipid bilayer phase transition should be included as a specification test whereas a statement on lines 127-128 indicates the entire set of characterisation tests that may be included as specification tests. There is no need to emphasize that this particular characteristic might be included as a specification test. Proposed change (if any): "characterisation~~/specification testing for~~ **of** lipid bilayer phase transition […]"	Accepted. The words '/specification testing for' have been deleted.
Lines 117 -118	4	Proposed change: Characterization/specification testing for lipid bilayer phase transition; temperature and/or liposomal "surface charge" **with relevance to its stability and release rate comparison;**	Not Accepted. Surface charge is, similar to size and lamellarity a characteristic of the liposome
Lines 117-118	8	It is recommended to separate the parameters as the first concerns the whole bilayer and the second only the surface: - lipid bilayer phase transition temperature	Comment noted. The bullet points have been separated.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		- liposomal surface charge (e.g., electrophoretic mobility or zeta potential) It should be considered to add internal sulphate and ammonium ion concentration in case of doxorubicin liposomes as the internal environment is relevant to keep doxorubicin in the precipitated state.	Internal sulphate and ammonium ion concentration in case of doxorubicin liposomes are already reflected in lines 126-127.
Lines 119-120	4	Comment: We would prefer if this statement was removed at this point in time because appropriate analytical tools do not exist to get the details of the physical state of the molecule such as peptide, antibodies, proteins, oligonucleotides etc.; if removal is not possible, add "if necessary" (see below) to this point. Proposed change: **"If necessary**, a confirmation of the physical state of the active substance inside the liposome (e.g. precipitation in the case of doxorubicin);"	Accepted. The proposal of the stakeholder was changed. The words 'If relevant' have been added.
Line 121	3	Comment: While it may be valuable to provide the fraction of drug that is surface bound, a more general characteristic should be recommended. Proposed change (if any): "~~fraction of drug that is surface bound~~ **distribution of drug within liposome (e.g. surface, bilayer, interior, *etc.*)**"	Accepted. The words 'fraction of drug that is surface bound' has been replaced by 'distribution of drug within liposome (e.g. surface, bilayer, interior, etc.)'
Line 121	4	Comment: "Fraction of drug that is surface bound": this point may not be very relevant from drug perspective as far as the release rate profile is maintained in context of its stability.	Comment noted. Line 121 has been changed to be more general according to the comment of

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			stakeholder 3.
Line 123	4	Proposed change: Details of linkage chemistry **(such as** PEG-lipid **or similar constructs with or without PEG);**	Accepted. The words '(such as PEG-lipid or similar constructs with or without PEG)' have been added.
Line 124	4	Proposed change : Molecular weight of pegylated lipid**, other conjugated lipids, and other molecular conjugates** and **their** size distribution;	Accepted. The words 'conjugated (e.g.' have been added.
Line 125	4	Proposed change: Disposition of PEG **or other lipid or molecular conjugate** at surface **as long as the release rate is consistent with drug product performance;**	Not accepted. Multiple parameters may have effects on the performance of the formulation not limited to the release rate.
Line 126	4	Proposed change: Stability of pegylation **with reference to drug product release rate comparability and stability;**	Not accepted. Multiple parameters may have effects on the performance of the formulation not limited to the release rate.
Line 129	4	Proposed change: **Reliable and** discriminating validated in-vitro release methods should be developed […]	Accepted. The words 'Reliable and' have been added.
Line 134	4	Proposed change: Monitor stability on storage, ~~and be sensitive to~~ ensure batch to batch consistency**, and also develop a functional bioassay at entry into human clinical development phase.**	Not accepted. Development of a functional bioassay at entry into human clinical development phase is no general requirement liposomal products developed with reference to an innovator liposomal product
Line 140	2	Comment:	Not accepted.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		The text reads 'extensive state of the art characterisation studies should be applied'. It seems unnecessary to use the term extensive as 'state of the art' is well known and well understood term. Proposed change (if any): Please change to '~~**extensive**~~ state of the art characterisation studies should be applied'.	The word 'extensive' should stress the need of a comprehensive comparability study. The wording is in compliance with other guidelines e.g. Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues (EMEA/CHMP/BWP/49348/2005)
Line 141	3	Comment: The word "quality" is misplaced in this sentence. Proposed change (if any): "[...] in order to demonstrate with a high level of assurance that the ~~quality is~~ **characteristics are** comparable"	Accepted. The words 'quality is' are replaced by 'characteristics are'.
Line 143	3	Comment: The work "quality" is misplaced in this sentence. Proposed change (if any): "suitable to adequately characterise ~~the quality of~~ the test and reference liposomal products".	Accepted. The words 'the quality of' have been deleted.
Line 144	8	Please add the following sentence in order to facilitate assessment: *The relevance of the selected tests for equivalent performance of the drug product in vivo should be discussed.*	Accepted. The words 'The relevance of the selected tests for equivalent performance of the drug product in vivo should be discussed.' have been added.
Between line 151 and 152	8	Please add the following sentence: *It is recommended to consider basic principles as outlined in the guideline on similar biological medicinal products (CHMP/437/04).*	Not accepted. The reflection paper refers to ICH Q5E where such comparability approaches are described.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
Lines 158-159	8	Comparative investigations should not only be considered as a consequence of changes to the manufacturing process during pharmaceutical development but also after marketing authorisation, e.g., scale up. NL therefore proposes to amend the sentence accordingly.	Accepted. The words 'but also after marketing authorisation (e.g. for scale up).' have been added.
Line 159	4	Comment: Please add sentence below. Proposed change: "[…] when a change is introduced into the manufacturing process during development. **Appropriate critical process parameters are identified to ensure consistent physico-chemical drug product characteristics**".	Not accepted. The proposed change is already addressed in other sections of the reflection paper.
Line 160	8	Comment: It may be clarified that the required in vivo studies may be either or both non-clinical and clinical. It may further be indicated that these required in vivo studies relate to pharmacokinetic or pharmacodynamic studies (i.e., no comparative clinical trials are indicated) Proposed change: In vivo **non-clinical pharmacokinetic or pharmacodynamic and/or clinical pharmacokinetic** studies may be necessary to demonstrate that the changes do not impact the safety and efficacy profile of the product when results from physicochemical testing indicate a change in the properties of the product.	Accepted. The corresponding non-clinical and clinical sections are restructured and considerably rewritten. It is believed that these sections now provide straightforward answers to the questions raised in the comment.
Line 166 – General Aspects	1	Comment: As this paper recommends non-clinical animal studies for	Not accepted. It is not necessary to repeat principles laid down in the corresponding ICH

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		comparability studies that cannot be evaluated by quality investigations alone, or by study in man (tissue-distribution), the insertion of the following comment should be considered within this section. Proposed change: The design of non-clinical studies is important in order to minimise the number of animals required in accordance with the 3R principles (reduce/ refine/replace). Considerations should include improved rationale/experimental design, avoidance of screening in irrelevant disease and use of appropriate time points and appropriate breadth of organ sampling.	guidelines here.
169-174	2	Comment: The text states: "In general, required non-clinical studies to be performed prior to bioequivalence testing should include comparative investigation of pharmacokinetics, tissue distribution, toxicology and pharmacological studies. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies can be reduced. Therefore, it may be decided on a case-by-case basis which studies could be waived." This section requires further clarification e.g. by means of a decision tree, in which cases the non-clinical study program might be reduced. In case pharmaceutical comparability between test and innovator product can unequivocally be proven, and the	Partly accepted. The paragraph has been reworded to improve clarity. Decision trees are indeed useful to communicate regulatory expectations. However, products under the scope of this guideline have rather different pharmaceutical, pharmacological and therapeutic properties. Developing a common decision tree which covers all possibilities would be a very difficult, if not impossible task. Publishing a decision-tree would be more appropriate in product specific notes than a general reflection paper.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		preclinical pharmacokinetic studies demonstrate equivalent behaviour with regard to pharmacokinetics and tissue distribution, pharmacodynamic studies could be waived. The request for PD studies seems to be a more strengthened approach in case the formulations differ in terms of e.g. qualitative composition. Proposed change (if any): "In general, ~~required~~ non-clinical studies to be performed prior to bioequivalence testing can ~~should~~ include comparative investigation of pharmacokinetics, tissue distribution, toxicology and pharmacological studies. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies can be reduced. Therefore, it may be decided on a case-by-case basis which studies could be waived. In addition, a decision tree should be included in the reflection paper.	
169-174	6	Comment: The text states: "*In general, required non-clinical studies to be performed prior to bioequivalence testing <u>should</u> include comparative investigation of pharmacokinetics, tissue distribution, toxicology and pharmacological studies. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies can be reduced. <u>Therefore, it may be decided on a case-by-case basis which studies could be waived</u>*."	Partly accepted. The paragraph has been reworded to improve clarity. Decision trees are indeed useful to communicate regulatory expectations. However, products under the scope of this guideline have rather different pharmaceutical, pharmacological and therapeutic properties. Developing a common decision tree which covers all possibilities would be a very difficult, if not impossible

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		We propose to include further clarification in this section, e.g. by means of a decision tree, in which cases the non-clinical study program might be reduced. We propose that in case pharmaceutical comparability between test and innovator product can unequivocally be proven, and the preclinical pharmacokinetic studies demonstrate equivalent behavior with regard to pharmacokinetics and tissue distribution, pharmacodynamic studies could be waived. The request for PD studies seems to be a more strengthened approach in case the formulations differ in terms of e.g. qualitative composition. Proposed change: "In general, ~~required~~ non-clinical studies to be performed prior to bioequivalence testing **can** ~~should~~ include comparative investigation of pharmacokinetics, tissue distribution, toxicology and pharmacological studies. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies can be reduced. Therefore, it may be decided on a case-by-case basis which studies could be waived. In addition, we propose to include a decision tree in the reflection paper for further clarification.	task. Publishing a decision-tree would be more appropriate in product specific notes than a general reflection paper.
182	1	Comment: A separate section dedicated to the requirements for analytical methods to be used in PK/tissue distribution	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		studies should be included based on regulatory experience gained to date. Proposed change: Addition of following text into a new section (section 2.2.2): For comparison to a reference liposomal product, analytical methods developed and validated to quantify encapsulated and unencapsulated drug in blood/plasma and unencapsulated drug in tissue will be necessary, in addition to traditional methods for total drug and metabolite in blood/plasma and tissues. Individual quantification of unencapsulated and encapsulated drug involves separation methodologies that require special attention to verify their reliability. For every blood/plasma sample, total drug levels should be quantified without separation of encapsulated and unencapsulated drug as an independent verification of the reliability of the separation methodology. While it might be feasible to quantify unencapsulated, encapsulated and total drug in blood/plasma, it is acknowledged that tissue processing is likely to disrupt liposomes. For unencapsulated drug in tissues, care should be taken to separate the unencapsulated drug prior to tissue processing steps that are likely to result in destruction of liposomes. Careful attention should be paid to the impact of	

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		all sample processing procedures during the course of method development, employing methodologies to verify the suitability and interpretability of all bioanalytical results. The methods of analysis used to quantify the levels of drug (total, unencapsulated and encapsulated) and metabolite in the plasma and tissues and their validation should be described. The lower limits of quantitation and recovery in plasma, tissues and, where relevant, in particular tissues of interest e.g. in tumours, should be stated.	
182	1	Comment: A new subsection should be included specifically for non-clinical tissue distribution studies and should be based on regulatory experience gained to date. Proposed change: Addition of following text into a new subsection titled “non-clinical tissue distribution studies” These studies provide pivotal evidence of the comparability of disposition of liposomal drug products, as it is not possible to have a full picture of the distribution in man from blood/plasma data alone. As such, the studies should be conducted to GLP standards in species relevant with respect to the pharmacology and safety of the product. Doses should be chosen to be relevant to the range of blood levels observed at therapeutic doses in man. The test	Partly accepted The section about non-clinical pharmacokinetic studies has been restructured. As a result, a separate subsection on distribution studies was not considered necessary.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		product should be produced using the final manufacturing process and would ideally be from the same batch used for the pivotal clinical studies. Sampling time points and sampling duration should be carefully selected so as to accurately quantify the time course of unencapsulated and total drug and metabolite in tissues balancing the need to quantify early drug release from liposomes (e.g. over first 15 min) and persistence of drug in particular tissues. As these studies involve destructive sampling, the number of animals to be included will depend on the number of sampling time points, between animal variability in distribution of drug to tissues and variability as a result of experimental procedures (tissue excision, weight, homogenisation and sampling as well as bioanalytical sources of variability). Careful selection of sampling times will increase the precision of derived parameters. Pilot studies to establish the appropriate dose levels, necessary sampling strategy and the number of animals to be included are advised to avoid failed or uninterpretable pivotal studies. Tissues for analysis should include those associated with the safety and efficacy of the drug as well as those involved in significant processing/elimination of liposomes. Analyte levels in tissue and blood/plasma should be expressed as a % of dose (taking into account tissue or blood weights or volumes).	

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		There is insufficient regulatory experience of such studies to support specific decision criteria for comparability of tissue distribution. Replicate study designs where at least the reference product is replicated are advised, as otherwise any differences between test and reference product are uninterpretable. A variety of data displays should be utilised including, but not limited to, PK parameter differences and ratios between treatments and visual comparisons of amount versus time profiles for each tissue and each analyte. The clinical implications of any noted differences in tissue distribution between test and reference product should be discussed.	
187-188	6	Comment: The text states: "*Single and multiple dose studies at different dose levels may be needed to support the claim of similar pharmacokinetics*." We propose that it should be decided on a case by case basis, depending on the drug product used, whether a study under single or multiple dose conditions should be performed. E.g. if the clinical application acc. to the product monograph of the drug product is intended for single dose administration only, no studies under multiple dose conditions should be required. Proposed change: "*Single **or** ~~and~~ multiple dose studies at different dose levels*	Not accepted. In certain cases both studies (single or multiple dose) might be needed. The proposed change would exclude this possibility.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		may be needed to support the claim of similar pharmacokinetics."	
188	2	Comment: 'The active substance concentration in tissues relevant to the toxicity and/or efficacy of the product should be determined and quantitatively compared with the reference liposomal product.' The EGA would favour the elaboration of clear general principles (eg, in the form of a decision tree) to determine when a separate determination of free and liposomal (or total) drug substance becomes necessary and when determination of total amount of drug substance is sufficient. Proposed change (if any): The EGA would appreciate if this section could elaborate further on the above topic. A simple decision tree might clarify.	Not accepted. If feasible, unentrapped concentrations should be measured in all cases.
188	6	Comment: The text states: "*Single and multiple dose studies <u>at different dose levels</u> may be needed to support the claim of similar pharmacokinetics*." We propose to add a clarification why different dose levels are required and how the selection of different levels should be done.	Accepted. The following sentence has been added to the text: "More than one dose is needed to characterize the nonlinear features of the pharmacokinetics. Doses should be chosen based on blood levels observed at therapeutic doses in man To establish the correct dose, the use of allometric equations or PBPK modelling is recommended
190-191	2	Comment:	Not accepted. See the comment at line 169.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		The text states: "The active substance concentration in tissues relevant to the toxicity and/or efficacy of the product should be determined and quantitatively compared with the reference liposomal product. " Proposed change (if any): Please insert a decision tree.	
190-191	6	Comment: The text states: "*The active substance concentration in tissues relevant to the toxicity and/or efficacy of the product should be determined and quantitatively compared with the reference liposomal product*. " We propose to add a clarification that for preclinical PK and distribution studies the total drug content should be measured. Proposed change: "*The* ***total drug*** ~~*active substance*~~ *concentration in tissues relevant to the toxicity and/or efficacy of the product should be determined and quantitatively compared with the reference liposomal product*. "	Partly accepted. It has been clarified that "The kinetics (including tissue distribution and excretion) of both the free drug and the encapsulated drug should be investigated if feasible."
191	8	Comment: Both the amount of free drug and of encapsulated drug are important pharmacokinetic parameters and have to be named in this part of the text. Proposed change (if any): addition of: "The kinetics (including tissue distribution and excretion) of both the free drug and the encapsulated drug should be investigated if feasible."	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
192 - 201	8	Comment: When the pharmacokinetic and in vitro studies have proven the similarity of the liposomal product, it can be assumed that there will be no difference in pharmacodynamics and toxicity of the drug. Proposed change (if any): Lines 192 – 201 should be substituted by: “Non-clinical pharmacodynamic and toxicological studies The non-clinical pharmacodynamic studies should include in vitro tests which characterize the interaction between liposomes and target cells or with other cells where the interaction is toxicologically relevant and important. In general in vivo pharmacodynamic and toxicity studies should not be needed, because when the pharmacokinetic and in vitro studies have proven the similarity of the liposomal product, it can be assumed that there will be no difference in pharmacodynamics and toxicity of the drug.”	Not accepted. While it is agreed that the ideal scenario would be to use in vitro pharmacodynamic studies in place of in vivo models the level of scientific understanding and development of such assays is limited at this time. With future developments in mind, the relevant section has been reworded to allow the possibility for the sole use of in vitro pharmcodynamic studies should a scientifically validated option exist. In relation to the need for toxicity studies, the revised wording of this section is considered to adequately reflect that in general toxicity studies may not be needed while outlining situations in which further investigations may be required.
196-197	6	Comment: The text states: “*in-vitro tests which characterize the interaction between liposomes and target cells or with other cells where the interaction is toxicologically relevant and important.”* We propose to add a clarification in the text that this criterion does only apply for active targeting liposomal drug formulations (e.g. antibody-conjugated liposomes using tumor-specific cell receptors as target). For passive drug targeting (e.g. using vessel permeability for	Not accepted. This guidance document relates to follow-up ("generic") liposome products. Antibody-conjugated liposomes are biological products which are outside of the scope of this guidance document.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		diffusion in tumour tissue), this is not deemed applicable. Proposed change: "*in-vitro tests which characterize the interaction between liposomes and target cells or with other cells where the interaction is toxicologically relevant and important.* ***These tests can be relevant for active targeting liposomal drug formulations (e.g. antibody-conjugated liposomal formulations)****."*	
198 -201	1	Comment: Organ function tests in non-clinical studies can be used to support equivalence in the context of known target organ toxicity. An example could be given to clarify what type of study could be appropriate. Proposed change: In general toxicity studies may not be needed~~.~~. ~~In~~However, depending on the outcome of pharmaceutical comparability investigations, and nature of any ~~the~~ toxicity produced by ~~of~~ the product, ~~the company may need to conduct appropriate toxicity studies.~~ appropriate organ function tests may be required to support equivalence in the context of known target organ toxicity e.g. in the case of suspected toxicity to the heart, a test of function such as an assessment of cardiac function by measurement of left ventricular end-diastolic pressure in a rodent model may be appropriate.	Accepted
198-201	6	Comment:	Partly accepted. This section has been

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		The text states: "*In general toxicity studies may not be needed, however depending on the outcome of pharmaceutical comparability investigations, and nature of the toxicity of the product, the company may need to conduct appropriate toxicity studies.*" We propose to specify under which conditions toxicity studies are not required. E.g. in case the test and reference product do not differ in qualitative composition and hence, pharmaceutical comparability between test and innovator product can unequivocally be proven, toxicity studies are not deemed necessary. Proposed change: "*In general toxicity studies ~~may not be needed, however depending on the outcome of pharmaceutical comparability investigations, and nature of the toxicity of the product, the company may need to conduct appropriate toxicity studies~~ are not needed in case of pharmaceutical comparability between test and innovator product."*	rewritten to include examples of where toxicity studies may be required, e.g specific organ function tests or immunotoxicity investigations.
205- 221	1	Comment: Some of the information in this clinical section is also applicable to the non-clinical considerations. Thus, it is suggested that the text be moved into the 'general aspects' section. Proposed change:	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Move text (lines 205-221) into 'General Aspects' section of the "Non-Clinical and Clinical Requirements" chapter.	
207	8	Comment: 'liposomal active substance' is somewhat confusing. Proposed change: The clearance of the ~~liposomal~~ active substance **given in a liposomal formulation** is dependent on:	Accepted
217-221	8	Comment: In certain conditions indeed the free fraction (so non-encapsulated and non-protein bound fraction) may be relevant to measure as well, e.g. in case protein binding is high. In that case, only part of the non-encapsulated fraction is considered active, and relatively small changes in 'total' non-encapsulated fraction may lead to relatively large and significant differences in free/active fraction of the active substance.	Agreed
220	8	Comment: the term 'free' may not be the same as non-encapsulated, since free constitutes the non-protein-bound subfraction of the non-encapsuled fraction, whereas non-encapsulated may be both the unbound and protein-bound subfraction of this non-encapsulated fraction. Proposed change: replace 'free' by 'non-encapsuled'.	Accepted. The word "free" might lead to misunderstanding; therefore the terms "unentrapped" or "unencapsulated" are used instead.
222-228	7	Comment: The text states: "*Pharmacokinetic behavior might be dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared in the*	Accepted. The sentence has been reworded.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		whole dose range unless linearity has been demonstrated in the recommended dose range or the most sensitive dose can be determined and justified. " We propose to clarify in this reflection paper how the most sensitive dose is to be determined for a liposomal product.	
223	5	Comment: This key point is often the case. Proposed change (if any): Replace "might" with "is often"	Accepted
223-226	2	Comment: 'Pharmacokinetic behaviour might be dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared in the whole dose range unless linearity has been demonstrated in the recommended dose range or the most sensitive dose can be determined and justified. If the product is administered at several doses for different therapeutic indications, a pharmacokinetic study with each particularly recommended dose is needed unless linearity has been demonstrated.' The EGA would propose that literature data be considered as sufficient to establish linearity. Proposed change (if any): Pharmacokinetic behaviour might be dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared in the whole dose range unless linearity has been demonstrated, by appropriate literature data, the recommended dose range or the most sensitive	Partly accepted. The sentence has been reworded as suggested. Otherwise see the comment at line 169.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		dose can be determined and justified.' If the product is administered at several doses for different therapeutic indications, a pharmacokinetic study with each particularly recommended dose is needed unless linearity has been demonstrated (by appropriate literature data). Proposed change (if any): Please insert a decision tree.	
222-228	2	Comment: "Pharmacokinetic behavior might be dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared in the whole dose range unless linearity has been demonstrated in the recommended dose range or the most sensitive dose can be determined and justified. If the product is administered at several doses for different therapeutic indications, a pharmacokinetic study with each particularly recommended dose is needed unless linearity has been demonstrated. " The decision which studies have to be performed in case of drugs exhibiting non-linear pharmacokinetic should be adjusted with regard to the general bioequivalence recommendations stated in the Guideline on the Investigation of Bioequivalence 2010. The amount of studies should not depend on different therapeutic indications. In addition, it should be clarified in this reflection paper how the most sensitive dose is to be determined for a liposomal product.	Partly accepted. The sentence has been reworded. Otherwise see the comment at line 222

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Please harmonise the proposal in line with the bioequivalence approach to IR products as adopted in 2010.	
228	4	Comment: Please add statement below. Proposed change: "[…] unless linearity has been demonstrated. **If a non-linear pharmacokinetic profile is demonstrated at equivalent doses even with the innovator product then an overall PK comparability equivalence and equivalent safety margin would be deemed sufficient**".	Accepted
230	5	Comment: This key point is often the case. Proposed change (if any): Open the sentence with "As may commonly be expected to be the case, if the product could not…"	Accepted.
230-232	2	Comment: 'If the product could not be administered to healthy volunteers, a pharmacokinetic study can be performed in patients. If a single-dose study is not feasible (i.e. active substance is not tolerable in healthy volunteers) multiple dose pharmacokinetic studies in patients are acceptable.' This section addresses design considerations in quite narrow terms.	Partly accepted. The sentence has been reworded.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): The text should be amended as follows: 'If the product could not be administered to healthy volunteers feasible ~~**(i.e. active substance is not tolerable in healthy volunteers)**~~, a pharmacokinetic study can be performed in patients. If a single-dose study is not feasible, multiple dose pharmacokinetic studies ~~**in patients**~~ are acceptable.'	
230-232	8	Comment: It should be indicated that multiple dose studies in patients are accepted when single dose studies in patients (instead of healthy volunteers) are not feasible. Non-tolerability and /or toxicity is the reason for not conducting studies in healthy volunteers, and this may be indicated. Proposed change: If the product could not be administered to healthy volunteers **(e.g. active substance is harmful for, or not tolerable in healthy volunteers)**, a pharmacokinetic study can be performed in patients. If a single dose study is not feasible **in patients** ~~(i.e. active substance is not tolerable in healthy volunteers)~~ multiple dose pharmacokinetic studies in patients are acceptable.	Accepted and the sentence is reworded.
235	2	"The validated bioanalytical method should reliably quantify encapsulated and non-encapsulated drug substance. " It should be stated more clearly (e.g. by means of a decision tree) in which cases the separate analysis of free and encapsulated drug is required and in which cases the analysis total drug is sufficient.	Not accepted. Unencapsulated concentrations should be measured in all cases unless it is proven unfeasible.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Proposed 234-change (if any): Please insert a decision tree.	
235-239	2	Comment: A decision tree identifying situations where metabolite(s) measurement would be required. Proposed change (if any): Please include a decision tree.	See the comment at line 169
238-239	7	Comment: The text states: *"If there are several metabolites then the choice of metabolite should be justified on kinetic grounds. If one or more metabolites have significant clinical activity then it might be required to compare their kinetics as well*." This criterion should be adjusted with regard to the Guideline on the Investigation of Bioequivalence 2010, which states that analysis of the metabolite is not necessary if it is possible to measure the parent drug (in case of liposomes: free and encapsulated drug) reliably. Acc. to the reflection paper, analysis of the metabolite may facilitate to assess and compare a release rate, since metabolism of the active substance takes place only after release from the liposomes. However, if it is possible to analyze the active substance (=free drug) reliably, additional analysis of the metabolite is deemed not necessary.	Not accepted. The pharmacokinetics of liposome products are essentially different from immediate release products covered in the cited Bioequivalence Guideline. In the case of liposomes, the distribution and elimination is controlled by product related factors and there is no absorption after i.v. administration. This is in contrast with orally administered immediate release products where the absorption depends on formulation related factors and the distribution and elimination is deemed to be formulation independent. This fundamental difference between the pharmacokinetics requires different regulatory considerations.
Lines 240-253	1	Comment: Information should be included here giving guidance on the importance of early sampling.	Accepted.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Proposed change: The following text could be inserted at Line 247: Early sampling timepoints, during and immediately after infusion of the product, should be included to ensure comparability with regard to early clearance by the reticulo-endothelial system.	
245-246	7	Comment: The text states: "*When relevant, rate and extent of excretion of active substance in urine should be compared*." It should be clarified in which cases the comparison of active substance in urine is required. Proposed change: We propose to add a clarification in which cases the analysis of urine might be required, but to emphasize that the analysis should generally be done in plasma or serum (as stated in the Guideline on the Investigation of Bioequivalence 2010 on page 4, section 1.1: "In bioequivalence studies, the plasma concentration time curve is generally used to assess the rate and extent of absorption".	Not accepted. This is considered to be a product specific issue which is outside of the scope of this guideline.
248	7	Comment: Partial AUCs: The clinical relevance for partial AUCs for a liposomal drug product should be addressed.	Not accepted Please note the definition of the partial AUC-s is not the same as recommended for modified release products. Using Cmax as a secondary bioequivalence

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
			metrics for intravenous products is not as straightforward as for tablets. Partial AUC-s are possible alternatives and there are several Partial AUC-s depending on the cut-off time. The clinical relevance of Partial AUC-s depends from the underlying PK-PD relationship and a general statement about their clinical relevance cannot be made.
250-251	7	Comment: The text states: "*Additionally, further descriptive parameters could be considered e.g. inter-compartmental clearance and volume of the peripheral and central compartments*." It should be specified in which cases these parameters should be presented.	Not accepted. These parameters are considered useful in all cases to support the claim of equivalent pharmacokinetics.
254	7	Comment: Acceptance criteria: We propose to - aAddress the possibility of scaled average bioequivalence approach - address criteria for steady state studies - add a clarification why bioequivalence should be established for Cmax and AUC0-t and additionally AUCinf. The parameters required for bioequivalence decision should be adjusted with regard to the general bioequivalence recommendations stated in the Guideline on the Investigation of Bioequivalence	Not accepted. The bioequivalence concepts used for orally administered products cannot be extrapolated for intravenously administered products with complicated distribution and elimination kinetics. For example the Guidance on Bioequivalence allows scaling for Cmax and Cmax is thought be characterizing the absorption process. But there is no absorption after intravenous absorption so the interpretation of this parameter is completely different.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		2010 (Cmax and AUC0-t required).	
254	7	Comment: “Acceptance criteria for the metabolite” It should be clarified in which cases the evaluation of bioequivalence for the metabolite is required and this criterion should be adjusted with regard to the general bioequivalence recommendations stated in the Guideline on the Investigation of Bioequivalence 2010, which states that analysis of the metabolite is not necessary if it is possible to measure the parent drug (in case of liposomes: free and encapsulated drug) reliably. It should also be clarified if the metabolite might be a surrogate in cases it is not possible to measure the free drug reliably (e.g. due to too low concentrations).	Not accepted. Setting criteria for metabolites requires case-by-case considerations. Several factors should be considered including whether the metabolite is biologically active or not or whether the metabolite can be accepted as a marker of the unentrapped concentrations or not.
254	2	Comment: It should be clarified in which cases the evaluation of bioequivalence for the metabolite is required and this criteria should be adjusted with regard to the general bioequivalence recommendations stated in the Guideline on the Investigation of Bioequivalence 2010.	
254-257	1	Comment: The following statement is supported. “Similarity should be demonstrated for the total, encapsulated and ~~non~~ unencapsulated drug. Generally, the 90% confidence intervals of Cmax, AUCinf and AUCt ratios should be within 80 - 125%”.	Agreed

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		Rationale: - Tighter limits (e.g. 90-111.11%) have not been required for generics of non-liposomal cytotoxics requiring demonstration of bioequivalence (e.g. capecitabine). It may be challenging for companies to power studies adequately to demonstrate comparable PK of the unencapsulated drug within 90-111.11%. - The use of the word 'generally' allows to keep the option open when considering whether to accept a PK study showing results just outside 80-125% but with convincing quality and non-clinical comparability results. Proposed change: None.	
255	7	Comment: The text states: "*Similarity should be demonstrated for the encapsulated and non-encapsulated drug. Generally, the 90% confidence intervals of […] should be within 80 - 125%.*" As the concentration of the free drug is expected to be very low and the variability of the free drug is expected to be high, the usual BE criteria should not be valid: As the free drug is expected to be mainly relevant for the toxicity of liposomal formulations, we propose that the acceptance criteria for this parameter to be as follows: the upper limit of the 90% confidence interval should not exceed the upper	Not accepted. Therapeutic equivalence can be questionable if the unencapsulated concentrations are significantly lower than that of the reference.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		bioequivalence acceptance limit 125% and a limit for the lower limit of 90% CI should not be required (which is in line with the requirement of Appendix II of the Guideline on the Investigation of Bioequivalence 2010, page 24 for Locally acting locally applied products). Proposed change (if any): *Similarity should be demonstrated for the encapsulated and non-encapsulated drug.* ***For the encapsulated drug*** ~~*Generally*~~*, the 90% confidence intervals of […] should be within 80 - 125%.* ***For the free drug, it should be demonstrated that the systemic exposure is not higher for the test product than for the reference product, i.e. the upper limit of the 90% confidence interval should not exceed the upper bioequivalence acceptance limit 125.00."***	
256-257	7	Comment: The text states that the acceptance criteria "…*might include partial AUCs*…" We propose to delete this requirement as it conflicts with line 248 stating "…*partial AUCs (e.g. 0-24h, 24-48h etc) should be evaluated descriptively."* Proposed change (if any): "*Additional metrics might include* ~~*partial AUCs, or*~~ *acceptance criteria for the metabolite*."	Not accepted. There is not any contradiction between carrying out a statistical hypothesis test and providing descriptive statistics.
259-262	7	Comment:	Partly accepted, the sentence has been

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		The text states: "*In general, the necessity for a clinical efficacy trial(s) besides the obligatory clinical pharmacokinetic studies is decided on a case-by-case basis depending on the sensitivity of the non-clinical models and clinical PK data to detect differences between innovator and the liposomal product developed with reference to it, and the complexity of the formulation*." We propose to delete this section. In case non-clinical and clinical PK study requirements are met, clinical efficacy studies should not be required. Proposed change (if any): ~~*"In general, the necessity for a clinical efficacy trial(s) besides the obligatory clinical pharmacokinetic studies is decided on a case-by-case basis depending on the sensitivity of the non-clinical models and clinical PK data to detect differences between innovator and the liposomal product developed with reference to it, and the complexity of the formulation."*~~	reworded to emphasize that proving therapeutic equivalence via comparative efficacy trials is not the preferred approach.
263	2	Comment: In the assessment of efficacy, the text states that "carrying out additional therapeutic equivalence studies are always required […]" Proposed change (if any): Considering a case-by-case approach, we propose to replace the sentence by the following one:"additional therapeutic	Partly accepted. Now the text states: "additional therapeutic equivalence studies are most likely required"

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		equivalence studies are likely to be required"	
263-268	8	Comment: It may be indicated stronger that clinical efficacy trials are often less sensitive to detect formulation dependent differences, and that for this reason non-clinical PK data and clinical PK data are preferred. Furthermore, the possibility for conducting non-clinical pharmacodynamic studies should be indicated. Proposed change: Carrying out additional therapeutic equivalence studies are always required if the formulations differ in terms of qualitative composition. As an example clinical studies including therapeutic equivalence studies might be required in cases when polymers are attached to lipids by means of different linking methods. **However, due to the relative insensitivity of clinical efficacy trials to detect formulation dependent differences, this is not the preferred approach. Therefore, w**hen developing a liposomal product with reference to an innovator product all attempts should be made to demonstrate equivalence of pharmaceutical quality of formulations and similarity in non-clinical **pharmacokinetic and pharmacodynamic** and clinical pharmacokinetic studies.	Accepted
269-273	9	Comment: Hypersensitivity, or infusion reactions to liposomes, which is known to be lethal in occasional hypersensitive, (mainly cardiac) patients, have been linked to their capability to activate the nonspecific, humoral arm of the immune system, called complement. There are numerous	Partly accepted. If it is necessary then the acute infusion reaction should be always investigated regardless of the target patient population.

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		papers (see list below, the most recent review on the subject attached) reporting complement activation by liposomes as the underlying cause of infusion-reaction-equivalent cardiopulmonary and hemodynamic changes. Several of these studies show direct correlation between complement activation and infusion reactions, providing rationale to measure the complement activating capability of liposomes in vitro, as a predictive laboratory marker of infusion reactions. In addition, several of these studies demonstrate substantial inter-species variation in sensitivity to liposome-induced infusion reactions, called complement activation-related pseudoallergy (CARPA), pointing to pigs as the most sensitive, best suited species to model the infusion reactions in *hypersensitive man, in whom the reactions are severe, life threatening*. Furthermore, several studies address the liposome characteristics contributing to complement activation and CARPA, pointing to size, charge, surface modifications (e.g., PEGylation), homogeneity, presence of aggregates and free drug, all contributing to the phenomenon in a very complex fashion. Consequently, individual specifications of the above variables and other relevant physicochemical parameters for both the liposomal product developed and the innovator may not be sufficient to provide assurance for identical immune (complement) reactogenicity; only direct in vitro and in vivo assays will give valid prediction of CARPA risk. For all these reasons I would like to suggest some modifications in the reflection paper as	

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		detailed below. Proposed change (if any): **Present version:** **Safety issues** Acute infusion reactions are relatively common with liposomal formulations. However, the frequency of such side effects is expected to be comparable unless the investigative products differ with respect to qualitative composition (e.g. different excipients). Use of animal models and unloaded (empty) liposomes for the investigation of hypersensitivity reactions may be necessary. **Final version:** Acute infusion reactions are relatively common with liposomal formulations. However, the frequency of such side effects is expected to be comparable unless the investigative products differ with respect to qualitative composition (e.g. different excipients) or production methods. However, it is recommended in this reflection paper that the qualitative and quantitative composition of the developed product should be identical or closely match the reference product. Still, to minimize the possibility of increased frequency of acute infusion reactions, use of in vitro and in vivo immune reactogenicity assays are required which are discussed in the toxicological studies section. If there is any sign that a new liposomal product might be associated with increased risk in this regard then the product development should be re-	

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		evaluated until reasons are clarified. Furthermore infusion reactions should be carefully evaluated in bioequivalence studies, and again, should any differences be noted, the product development should be re-evaluated. It is not anticipated that full-scale clinical trials are necessary prior to authorisation, however the clinical safety of similar liposomal products should be closely monitored in accordance with current EU legislation and pharmacovigilance guidelines.	
272-273	1	Comment: The need for investigating reactions to unloaded liposomes is not considered necessary. Rationale: Empty liposomes are not necessarily representative of loaded liposomes, and thus not relevant for investigation of hypersensitivity reactions. Proposed change: Animal models ~~and unloaded (empty) liposomes~~ may need to be used to investigate ~~for the investigation of~~ hypersensitivity reactions	Accepted with some rewording
272-273	4	Proposed change: Use of **appropriate** animal models and unloaded (empty) liposomes for the investigation of hypersensitivity reactions may be necessary **to assign the extent of potential adverse event.**	Accepted
270-273	5	Comment: The acute infusion reactions may also be	Accepted

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		exacerbated by difference in particle size distribution, and not just for differences in qualitative composition. Proposed change (if any): add the text: "…composition (e.g. different excipients) or production methods and controls (e.g. differences in particle size distribution)".	
272	7	Comment: The text states: "*However, the frequency of such side effects is expected to be comparable unless the investigative products differ with respect to qualitative composition (e.g. different excipients). Use of animal models and unloaded (empty) liposomes for the investigation of hypersensitivity reactions may be necessary*. " We propose to specify more clearly that the use of animal models and unloaded liposomes for the investigation of hypersensitivity reactions are only deemed necessary in case the investigative products differ with respect to qualitative composition. Proposed change (if any): "*However, the frequency of such side effects is expected to be comparable unless the investigative products differ with respect to qualitative composition (e.g. different excipients).* ***In this case, u****~~U~~se of animal models and unloaded (empty) liposomes for the investigation of hypersensitivity reactions may be necessary*. "	Not accepted. There are other factors besides the composition of the liposome which can cause acute infusion reaction.
274-278	7	Comment:	Not accepted. The request is noted, but so far

Line number(s) of the relevant text	Stakeholder number	Comment and rationale; proposed changes	Outcome
		The text states: "*The experience with liposomal formulations developed with reference to an innovator is limited. As only rather general recommendations can be given in this reflection paper companies are advised to seek product-specific scientific advice regarding specific questions on the data requirements for demonstration of comparability of liposomal formulations.*" Product-specific requirements should be published with a Question & Answer Paper in order to facilitate generic development of liposomal drug formulations.	product-specific requirements have been published only for biosimilar products.