Notice to applicant

Non-clinical and Clinical Studies Assessment for Liposome Drug Products & Nano similar Liposome Drug Products

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1. Introduction

Development of drug delivery systems to improve disease-specific targeting, to control drug release rates and/or to produce a formulation suitable for clinical use is desirable. One of the strategies have been encapsulation of the active substance(s) in the aqueous phase of a liposome, or incorporation or binding to the lipid components. 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. Release of drugs from liposome formulations, among other characteristics such as liposomal clearance and circulation half-life, can be modified by the presence of polyethylene glycol and/or cholesterol or other potential additives in the liposome. So, liposome drug products are designed to improve the stability of encapsulated active substances in vivo, the pharmacokinetics (including tissue distribution profile) of the active substances, and intracellular behavior of the active substances.

2. Scope

This document mainly provides information regarding nonclinical and early clinical studies of liposomal drug products and nano-similar liposomal drug products from regulatory point of view by identifying the points to be considered in the development of liposome drug products. (*N.B* Regarding the requirements for intravenous liposomal products developed with reference to an innovator liposomal product (Nano-Similar) refer to Annex I)*
Liposome drug products described in this document are also subject to other relevant notifications and guidelines. The active substances mentioned here include a low-molecular-weight chemical entity, a nucleic acid or a biological or biotechnological entity, including, for example, peptides and proteins.

3. General pre-Clinical and Clinical Consideration for the Development of Liposome Drug Products:

3.1. General Aspects:

Significant changes in pharmacokinetic characteristics can occur when an active substance is administered as a liposome drug product from those of the active substance administered by itself (i.e., changes in distribution volume and clearance, extension of the half-life, or a change in in vivo distribution may occur). Consequently, significant differences not only in the pharmacokinetic characteristics but also in the efficacy and safety of the active substance can be observed when the active substance is administered as a liposome drug product.
The rate and location of in vivo active substance release is a crucial parameter, which often determines the pharmacological effect and safety. An attempt should be made to develop the necessary methodology to understand the active substance release profile.

Nonclinical studies should be conducted using a well-characterized liposome drug product equivalent to the drug product for clinical use, and the release rate of active substance and product stability should be known under the chosen test conditions.

- Applicant is recommended to consult EDA (Egyptian Drug Authority) for Innovative drug application (New liposomal system and new molecule.
- Applicant should consider recommendations in this guidance during drug development for liposome drug products in conjugation with recommendations from international product-specific guidance.
- Applicant should also consider this guidance for generic liposomes development (Nano-similars) for bioequivalence and information necessary to demonstrate pharmaceutical equivalence

3.2. Non-Clinical Studies:

3.2.1 Non-clinical pharmacodynamics

After being delivered to the tissue, the liposome usually exhibits the PD (pharmacodynamics) response through the following processes: the liposome is incorporated into the cells and then releases the active substance, or the active substance is extracellularly released from the liposome and then incorporated into the cells. Generally, The PD studies should include demonstration of PD response in appropriately justified in vitro (where possible) and in vivo models. The development of in vitro tests capable of characterizing any interaction between liposomes and target cell are encouraged. If a ligand (targeting moiety) or antibody is conjugated to the liposome surface, the pharmacological action derived from the ligand (targeting moiety), or antibody should be determined in addition to the affinity to the target cells.

In vivo evaluation should involve an appropriate route of administration, justified dose levels, and a justified dosing regimen, depending on the proposed clinical application. Appropriateness of the pharmacological model should be discussed in respect of the pharmacokinetic behavior of the liposome drug product, as well as the pharmacokinetics and pharmacodynamics of the active substance when administered by itself.

The chemical composition and physicochemical properties (including size, surface charge, and the release rate of the active substance) of a liposome drug product affect pharmacodynamic properties. Some important factors to consider when designing studies to discuss the mechanisms of action include:

- The location and rate of in vivo active substance release.
- The binding of the liposomes to the target cells if a ligand (targeting moiety) or antibody is conjugated to the liposome surface.
- The intracellular fate of the liposomes (including lipids or other components) following cellular entry by endocytosis or other mechanism if the intracellular release of the active substance plays an important role in exhibiting the pharmacodynamic effect.

N.B*: *Failure to use both in vitro and in vivo models to assess the PD effects of the liposomes should be extensively justified using the evaluation method and the result by the applicant.

3.2.2 Non-clinical Pharmacokinetics:

The pharmacokinetic behavior of a liposome drug product can be largely different from that of the active substance administered in a non-liposomal form, and this difference may have a remarkable impact on the efficacy and safety of the product. therefore, it is important to compare the in vivo pharmacokinetics of the active substance administered by itself and the liposome drug product.

In general, the PK (pharmacokinetics) characteristics of the liposome drug product could be dependent on:

- Clearance of the liposome encapsulating active substance.
- Release rate of the encapsulated active substance from the liposome.
- Distribution of the liposome (changes in organ and/or tissue distribution and the amount of distributed active substances).
- Interaction of the liposome or active substance with plasma or serum protein, blood cells, or vascular endothelium.

When the in vivo pharmacokinetics and active substance release are investigated, the selection of animal species and animal model should be justified, with careful consideration of the following points: the expected clinical application of the liposome drug product, liposome composition, the properties of the active substance, and blood concentration and tissue distribution including the accumulation and retention in the target organ and/or tissue of both the active substance and liposome drug product.

These studies provide pivotal evidence of liposome 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 in accordance with the principles of Good Laboratory Practice (GLP).

If ligands (targeting moiety) or antibodies are conjugated to the liposome surface to provide targeting delivery, the animal species and model should be selected considering the differences in the expression and distribution of the receptor or epitope between the selected animal species and humans. As the quality attributes of liposomes such as the size, surface charge, morphology, and surface modification with a ligand (targeting moiety) or antibody may affect the in vivo distribution of a liposome drug product, the impact of variations in such properties on the in vivo distribution should be assessed. Investigating the relationship between the quality attributes and in vivo distribution will help justification of the product specifications in the future. In addition to the recommendations in the ICH S3 (S3A and S3B), S6(R1), and M3(R2) guidelines, the following factors are important in assessing the liposome drug product:

- It is useful to explain the purpose and significance of the liposome formulation by

comparing the pharmacokinetics of the liposome drug product and the active substance administered by itself.

- The appropriate pharmacokinetic parameters such as the Cmax, Area under the curve (AUC), and half-life of the total active substances and unencapsulated active substance in the blood, plasma, or serum should be analyzed, and changes in the pharmacokinetics of the active substance due to the liposome formulation should be discussed.
- The pharmacokinetic parameters should be measured at different dose levels and at appropriate time points.
- Distribution of the liposome drug products in organs and/or tissues relevant to proposed clinical use and route of administration should be evaluated. Specifically, total amounts of active substance in organs and/or tissues are required. A distribution time profile should be obtained using adequate sampling time points and sampling duration to accurately quantify the time course of the active substances.
- Some factors should be considered for the sampling schedules, such as sampling time points and sampling duration (e.g., the liposome stability after administration, and the profile of localization to specific organs and/or tissues). Samples taken in the initial distribution phase (e.g., <15 min) are considered informative for calculating. the distribution volume to estimate the stability of liposome in blood circulation (i.e., stability related to the initial burst of the liposome).
- If data on the concentration of the unencapsulated active substance in the relevant organs and/or tissues regarding the safety and efficacy of the liposome drug product are not available on account of difficulties in the analytical technique, attempts to measure the metabolites are useful.
- Study design details such as sampling method and sampling time points will affect precision of derived parameters. The appropriate dose levels, necessary sampling schedule, and the number of animals should be carefully determined.
- It is desirable to analyze the distribution of liposome drug product in organs and/or tissues associated with the safety and efficacy of the liposome drug product, as well as those involved in major metabolism and elimination of liposomes. Organs with safety concerns include the reticuloendothelial system, important organs related to clearance, and organs with accumulation potential (e.g., liver, spleen, kidneys, bone marrow, lungs, and heart), as well as organs protected by a blood-tissue barrier (e.g., the brain and testes).
- Measurement of active substance metabolites in blood, plasma, or serum (and the organs and/or tissues, if possible) is especially important when the metabolite is acknowledged to be the primary active compound. If one or more metabolites have substantial clinical activity, it is recommended to compare their pharmacokinetics and, where necessary, toxicokinetic, to determine accumulation following multiple doses.
- It may also be important to consider the protein and cellular interactions of

intravenously administered liposome because this factor is known to have the potential to influence the distribution, stability, and safety of liposome drug products. It is also useful to understand the pharmacokinetic behavior of the liposome drug product using an appropriate animal model and imaging technique (e.g., fluorescent labelling technique.)

- A ligand (targeting moiety) or antibody on the liposome surface can have a substantial impact on the tissue distribution and intracellular distribution of the liposome. It should be noted that these modifications can change the accumulation of liposome drug product, not only in target organs and/or tissues, but also in the other organs and/or tissues.
- The metabolic and excretion pathways of the active substance of a liposome drug product should be evaluated, because such an evaluation is linked to the safety and efficacy evaluation of the drug product. If a liposome component is predicted to affect safety, the distribution, metabolic, and excretion pathways of the component should be evaluated, where necessary.

3.2.3 Safety pharmacology

For liposome drug products (e.g., those that fall outside the scope of ICH S9 and require the safety pharmacology evaluation), safety pharmacology studies should be conducted in accordance with ICH M3(R2), ICH S7A, and ICH S7B, and in consideration of the Section 3.2.4.

3.2.4 Toxicology

In principle, the nonclinical evaluation of toxicities of liposome drug products should be equivalent to the evaluation for drug with new active ingredients. The toxicity studies of the liposome drug product should be conducted to assess the toxicological profile and exposure-response relations according to the ICH safety guidelines and M3(R2) guideline in Consideration of the following points:

- If a toxicity evaluation of the active substance administered by itself has already been completed, the toxicity of the liposome drug product using the same clinical route of administration as the active substance administered by itself should be evaluated by means of a short-term repeated-dose toxicity study using the intended clinical route of administration in one animal species. The obtained toxicity profile and toxicokinetic data should be compared with those of the active substance administered by itself. Based on the results, studies necessary for toxicity evaluation of the liposome drug product should be conducted from the generally conducted toxicity studies for drugs with new active ingredients.
- When the active substance is novel and toxicity and toxicokinetic data are unavailable, toxicity and exposure evaluations should be performed for the liposome drug product.

- Based on the ICH non-clinical safety guidelines. This will allow identification of particle-dependent toxicities and particle-dependent shifts in the encapsulated drug toxicity.
- When the active substance is likely to be present in blood circulation in the unencapsulated form, it may be necessary to perform repeated-dose toxicity studies of the active substance alone in appropriate animal species, using the intended clinical route of administration, and to compare the obtained toxicity and toxicokinetic data with those of the liposome drug product.
- Safety evaluation of the liposome components can be performed with the complete drug formulation (the whole liposome drug product) if the intention is to have the components approved exclusively for that drug product However, a toxicity evaluation of the components alone may be required when a suitable toxicity evaluation cannot be performed by using the whole liposome drug product only (e.g., because of novel toxicity concerns derived from the lipid structure or the potential for accumulation of the liposome components).
- According to selection of the relevant animal species, studies suggest that in laboratory animals (mice, rats, rabbits, guinea pigs and dog) and man macrophages in spleen and Kupffer cells in liver are primarily responsible for sequestration of nanoparticles, where as in some of the larger animals (pig, sheep, goat and cat), pulmonary intravascular macrophage (PIM) are mainly involved in trapping/ sequestration.
- If toxicity is observed, analysis of results should indicate that the occurrence of toxicity is unrelated or correlated with active ingredient and/or nanocarrier. Based on the unique biodistribution of the liposome drug product, additional organ specific toxicity studies may be required.
- In cases where the liposome drug product does not show any clinically significant pharmacokinetic difference compared to, traditional / conventional drug / active substance and has no difference in biodistribution, exemption of some toxicity studies may be given on the basis of ‘case-by-case approach’.

3.2.5 Toxicokinetic

In addition to blood, plasma, or serum concentration, measurement of the active substance in the target organs and/or tissues and toxicologically relevant organs and/or tissues is useful for toxicity evaluation of liposome drug products.

3.2.6 Additional studies

Depending on the physicochemical and/or pharmacokinetic characteristics of the liposome drug product and/or the lipids used for its manufacture, histological and functional evaluation of target organs may be necessary.
Acute infusion reactions are relatively common with liposome drug products. The use of in vitro and in vivo studies such as complement activation assays (and/or macrophage/basophil

activation assays) and studies in appropriate animal models should be considered to evaluate the potential adverse events.
Studies to investigate hematotoxicity, antigenicity, and/or immunotoxicity (ICH S8) should be considered depending on the characteristics of the liposome drug product, including the characteristics of the liposome or the pharmacological properties of the active substance.

3.3 Clinical Studies:

3.3.1 Considerations for first-in-human studies

Liposome drug products are often designed to influence the stability of encapsulated active substances in vivo, the pharmacokinetics (including tissue distribution profile) of the active substances, and intracellular distribution of the active substance. Therefore, in addition to the information recommended in the ICH S3 (S3A and S3B), S6(R1), M3(R2), and the PFSB/ELD Notification No. 0402-1 “Guidance for establishing safety in first-in-human studies during drug development” (dated April 2, 2012), when considering the first-in-human studies, it will be essential to consider information specific to the liposome drug product (e.g., nonclinical pharmacokinetic data of the liposome drug product and the active substance, proposed clinical use, and route of administration).
-In a nonclinical pharmacokinetic study, the time course of liposome drug products for the total active substance, unencapsulated active substance, and metabolites (and encapsulated active substance, depending on the properties of the liposome drug product) should be quantified before first-in-human studies conducted using pharmacokinetic parameters, sampling time points and durations that have been carefully selected, as follows:

- Pharmacokinetic parameters such as Cmax, AUC, and half-life, both for the total active substances, and for unencapsulated active substances in the blood, plasma, or serum.
- A sufficient number of samples should be collected to adequately describe the plasma concentration-time profile. Frequent sampling at early time points is considered useful for providing reliable information about the initial distribution process. In general, the sampling schedule should be designed to provide a reliable estimate of the total extent of exposure.
- Distribution of liposome drug products in target lesions and major organs. During evaluation, the total amount of the active substance in the target lesion and major organs should be measured at the time points that enable the estimation of the plasma concentration time profile over an adequate period of time.

-The starting dose for first-in-human studies should be chosen in compliance with ICH M3(R2) and “Guidance for establishing safety in first-in-human studies during drug development,” and by considering all related nonclinical data, including critical product Attributes, pharmacological dose-response, pharmacokinetics, and pharmacological/ toxicological profile.

-Dose-limiting toxicity in humans can be determined in a similar way to that of conventional drugs, except for hypersensitivity reactions, because these reactions are not always dose dependent.
-Potential critical quality attributes for each liposome drug product should be identified and used to evaluate consistency. Consistency of the quality attributes should be confirmed between the products used for the first-in-human studies and those for nonclinical studies, and test procedures should be established before the commencement of first-in-human studies.
-The stability of the liposome drug product must be ensured throughout the first-in- human studies by using the stability test.
-If the manufacturing process (including the scale-up) used to prepare a liposome drug product for nonclinical studies is changed before the first-in-human studies are conducted, comparability should be demonstrated.

3.3.2 Clinical Pharmacology Studies

a. Pharmacokinetic and Mass Balance Studies for Liposome Drug Products

Information from pharmacokinetic studies is useful for establishing dosing regimens and developing dose-concentration-response relationships. The study design should be based on the anticipated dosing regimen in the intended patient population. We recommend using a population pharmacokinetics approach, where appropriate.23
The pharmacokinetic measures or parameters should include area under the plasma concentration versus time curve (AUC), peak plasma concentration, time to peak plasma concentration, elimination half-life, volume of distribution, total clearance, renal clearance, and accumulation for both free and total drug, as appropriate. For mass balance studies, you should collect and assay blood (i.e., plasma or serum, as appropriate), urine, and fecal samples for the radiolabeled moiety. For these studies, you should monitor and quantify both parent drug and any metabolites present, as appropriate.
You should determine major metabolites associated with the therapeutic and toxic effects of the drug substance.
We also recommend conducting the following in vivo studies:
i. Multiple-dose study evaluating the drug pharmacokinetics after administration of the liposome drug product.
ii. Dose-proportionality study over the expected therapeutic dose range of the liposome drug product.
iii. Exposure-response studies if available.

-Depending on the target patient population and the proposed therapeutic indication for the drug, you should consider conducting drug interactions studies in specific populations.

B. Comparing Liposome Drug Products with Non-liposome Drug Product in Clinical Pharmacology Studies:

- **General Aspects:**

The documentation required to support regulatory approval of a liposomal formulation developed with reference to an innovator product should be detailed enough to warrant the conclusion of equivalent efficacy and safety compared to the innovator product. In general, the non-clinical studies to be performed prior to clinical studies should include comparative investigation of pharmacokinetics (including tissue distribution), toxicology and pharmacodynamics. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies could be reduced and if appropriate, it may be decided on a case-by-case basis which studies could be waived.

The drug disposition and pathways of elimination (including distribution, metabolism and excretion) as well as several important pharmacokinetic measures (Cmax, AUC) and parameters (e.g., clearance, volume of distribution, half-life) of a liposome formulation are likely to be different than those of a non-liposome formulation given by the same route of administration. For example, a liposome drug formulation may exhibit extended-release characteristics in comparison to a non-liposome formulation with the same active pharmaceutical ingredient.

If non liposome formulations have been approved, we recommend comparing the proposed liposome to the corresponding approved non-liposome formulation to elucidate differences in absorption, distribution, metabolism, and excretion (ADME). Conducting a mass balance study of a drug substance labeled with a radioactive isotope (e.g., 14C, 3H) in a liposome formulation and in a non-liposome formulation can be helpful in comparing drug ddistribution in organs of interest.

You should conduct comparative studies to define and assess differences in ADME of the active ingredient between liposome and non -liposome drug products when the following apply:

i. Two products have the same active ingredient.
ii. Two products are given by the same route of administration.
iii. The non-liposome drug product is approved and available for comparison.

In a single dose pharmacokinetic study, you should compare the liposome and non-liposome drug products using either a crossover or parallel study design that employs an appropriate number of subjects considering the study drug, disease for which it is used, use in specific populations, and other factors that apply. Depending on the drug substance under investigation, different doses of liposome and non-liposome drug products may be appropriate.

4. References:

1-EMA. (2013). Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product.

2-FDA. (2018). Liposome drug product guidance for industry.

3-MHLW, J. (2016, March). Guideline for the Development of Liposome Drug Products.

4-ICH Note for Guidance on Toxicokinetic: The Assessment of Systemic Exposure in Toxicity Studies S3A [July 2, 1996, PMSB/ELD Notification No.443]

5-ICH Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies S3B [July 2, 1996, PMSB/ELD Notification No.442]

6-ICH Duration of Chronic Toxicity Testing in Animals (Rodent and Non rodent Toxicity Testing) S4 [April 5, 1999, PMSB/ELD Notification No.655]

7-ICH Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1)[March 23, 2012, PFSB/ELD Notification No.0323-1]

8-ICH Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2) [February 19, 2010, PFSB/ELD Notification No.0219-4]

9-Guidelines for Non-clinical Pharmacokinetic Studies [June 26, 1998, PMSB/ELD Notification No. 496]

10- Clinical Pharmacokinetics Studies on Drugs [June 1, 2001, PMSB/ELD Notification No. 796]

11-Guidance for Establishing Safety in First-in-Human Studies during Drug Development [April 2, 2012, PFSB/ELD Notification No. 0402-1]

12- Guidelines for Evaluation of Nano-pharmaceuticals in India Government of India New Delhi October 2019

5. Annex I

Data requirements for intravenous liposome products developed with reference to an innovator liposome product (Nano-Similar):

-Introducing a novel class of intravenous liposome products present a promising frontier in drug delivery, leveraging lipid-based nanotechnology to enhance therapeutic efficacy and minimize adverse effects. In the pursuit of developing these innovative medications, a critical aspect lies in understanding the intricate data requirements, particularly in reference to established innovator liposome products. This exploration delves into the essential considerations and requirements governing the development of 'Nano-Similar' intravenous liposome products, elucidating the pivotal role of comprehensive data in ensuring safety, efficacy, and bioequivalence.

-Even for cases of ostensibly identical composition, variation in production and product and process control technology can lead to products with different therapeutic performance. The complete characterization of the stability, pharmacokinetics (including tissue distribution) of a new liposome product is critical to establish safe and effective use. This is because differences between the applicant’s product and innovator product regarding manufacturing process steps and formulation may substantially modify efficacy/safety due to changes in specific liposome-cell interactions and liposome distribution characteristics which are not detectable by conventional bioequivalence testing alone.

-The aims for developing the innovator and the evidence supporting its use should be considered when designing the non-clinical and clinical program for the liposomal products developed with reference to that innovator.

-However, the complexity of the liposomal formulation will determine whether comparative non-clinical studies could be reduced and if appropriate, it may be decided on a case-by- case basis which studies could be waived.

-In the comprehensive evaluation of the new liposomal product the body of evidence obtained in quality, non-clinical and clinical studies must be considered as a whole. If e.g. any relevant differences are found in non-clinical studies for the liposomal formulation developed with reference to the innovator then critical re-assessment of physio-chemical characteristics of the product is advised in order to clarify possible explanations for such differences before proceeding with clinical investigations. Differences between the innovator and the test product in the data generated to support product similarity would negate the similarity approach and could be a source of serious regulatory concern.

A) Non-Clinical Studies:

❖ Non-clinical pharmacodynamic studies:

The non-clinical pharmacodynamic studies should include:

-Where possible the development of in-vitro tests capable of characterizing an interaction between liposomes and target cells or other cells where the interaction is toxicologically relevant is encouraged. While it is possible to characterize the pharmacodynamic profile from such studies alone, it is recognized that the current state of knowledge on in vitro tests is limited and it is highly likely that in vivo studies will be needed at present.

-Demonstration of the similarity in pharmacodynamic response using appropriate in-vivo models and at various dose levels chosen considering the sensitivity of the model.

❖ Non-clinical pharmacokinetic studies:

-Some pharmacokinetic aspects of liposomal products regarding their performance in humans can be predicted by animal and, where applicable, cell-based models. However, the choice of appropriate species and models to investigate the in-vivo release of the drug from liposomes should be justified and distribution. In addition to the systemic exposure, similarities in the distribution and elimination should be demonstrated. 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 in accordance with the principles of Good Laboratory Practice (GLP) in species relevant with respect to the pharmacology and safety of the product.

-The test 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. If due to analytical reasons free concentrations cannot be measured, then attempts should be made to compare the metabolite concentrations in the target organs. 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, homogenization 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.

-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. The use of an appropriately selected internal standard should be considered to decrease the variability of the results. A variety of data displays should be utilized including, but not limited to, PK parameter differences and ratios between treatments and visual comparisons of amount vs time profiles for each tissue and each analyte. All estimates and data displays should include quantification of uncertainty, e.g. confidence intervals. The clinical implications of any noted differences in tissue distribution between test and reference product should be discussed.

- ***Analytes to be measured***

The kinetics (including tissue distribution and excretion) of both the unencapsulated drug and the encapsulated drug should be investigated if feasible.

❖ Non-clinical Toxicological studies:

In general, toxicity studies may not be needed. However, depending on the outcome of pharmaceutical comparability investigations, and nature of any toxicity produced by the product, 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.

Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudo allergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event.

Note:

If any relevant differences are found in non-clinical studies for the liposomal formulation developed with reference to the innovator, then critical re-assessment of physico-chemical characteristics of the product is advised to clarify possible explanations for such differences before proceeding with clinical investigations. Differences between the innovator and the test product in the data generated to support product similarity would negate the similarity approach and could be a source of serious regulatory concern.

B) Clinical Studies:

❖ Comparative pharmacokinetic studies:

- ***Dose to be investigated.***

-Pharmacokinetic behavior is often dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared over the recommended dose range unless linearity has been demonstrated. Demonstration of such linearity with encapsulated, unencapsulated, as well as total drug substance, would be required unless appropriate literature data are provided.
- In the case of non-linearity, demonstration of bioequivalence at the highest and lowest doses would suffice even if different doses were used for different indications. In such cases further clinical studies are not needed.
In some cases, bioequivalence studies cannot be carried out with certain doses due to ethical or other reasons. In these cases, assessment of therapeutic equivalence in each indication requires individual consideration.

- ***Design considerations***

It is probable that the active substance might not be tolerated by healthy volunteers. In such a case, a pharmacokinetic study may be performed in patients.
If a single-dose study is not feasible in patients, then multiple dose pharmacokinetic studies in patients may be acceptable.

- ***Analytes to be measured***

The validated bioanalytical method should reliably quantify total, encapsulated and unencapsulated drug substance. Since metabolism of the active substance takes place only after release from the liposomes, quantification of at least one metabolite regardless of its pharmacological activity may facilitate the assessment and comparison of active substance release rate from the liposomal formulation. 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 necessary to compare their kinetics as well.

- ***Pharmacokinetic parameters to be measured and reported***

The evaluated pharmacokinetic characteristics of total encapsulated and unencapsulated drug substance should be compared to allow assessment of the rate at which the active substance is released from the liposomes, since this will determine the onset and duration of the therapeutic effect. However, conventional pharmacokinetic metrics such as AUC and Cmax might not give sufficient indication of the rate of release at the target sites. Therefore, evaluation of additional pharmacokinetic parameters should be provided to describe other pharmacokinetic processes such as distribution and elimination in addition to rate and extent of release. When relevant, the rate and extent of excretion of the

active substance in urine should be compared. Early sampling time points, during and immediately after infusion of the product, should be included to ensure comparability regarding early clearance by the reticulo-endothelial system.
When the elimination rates of the unencapsulated and encapsulated active substance is different, that is for liposomes which release the active substance over a longer period, then additional pharmacokinetic parameters are needed such as clearance, volume of distribution, terminal half-life and partial AUCs (e.g., 0-24h, 24-48h etc). These parameters should be evaluated descriptively. This may enable further characterization of the integrity of liposomes and their uptake by peripheral tissues/reticuloendothelial system. Additionally, further descriptive parameters could be considered e.g. inter-compartmental clearance and volume of the peripheral and central compartments. It is recommended that the ratio of unencapsulated to encapsulated drug concentration overtime should be determined.

- ***Acceptance criteria***

Similarity should be demonstrated for the total, encapsulated and unencapsulated drug. Generally, the 90% confidence intervals of Cmax, AUCinf and AUCt ratios should be within80 - 125%. In special cases, additional metrics might include partial AUC, or acceptance criteria for the PK parameters of the metabolite.

- ***Assessment of efficacy:***

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 ability 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. It is highly likely that additional therapeutic equivalence studies will be 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, when 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 pharmacodynamics and clinical pharmacokinetic studies.
Differences between the innovator and the test product in the data generated to support product similarity would negate the similarity approach and could be a source of serious regulatory concern.

- ***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) or production methods. However, it is recommended that the qualitative and quantitative composition of the developed product should be identical or closely match the reference product.

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-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. Not limited to acute infusion reactions, the safety of liposome drug products must be compared based on the limited nonclinical and clinical data. Therefore, it is important to continue risk management efforts where necessary, even after marketing has begun.

It is not anticipated that full-scale clinical trials are necessary at the time of authorization, however the clinical safety of similar liposomal products should be closely monitored in accordance with current pharmacovigilance guidelines.