13 December 2018
EMA/CHMP/681372/2018
Committee for Medicinal Products for Human Use (CHMP)

Overview of comments received on 'Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance' (EMA/CHMP/800775/2017)

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

Stakeholder no.	Name of organisation or individual
1	Socratec-Pharma, Prof. Henning Blume, PhD
2	TLC Biopharmaceuticals B.V. (Taiwan Liposome Company)

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
2	**Foreword** Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius* and is highly effective against a spectrum of malignancies including both haematological and solid tumours. Its mechanism of action is through nucleotide base intercalation in double stranded DNA, and by inhibition of DNA topoisomerase II, an enzyme involved in DNA replication, which exhibits markedly increased activity in proliferating cells. It is also thought to generate free radicals, which lead to damage of cell membranes, DNA and proteins.[1] Doxorubicin hydrochloride was developed in the 1970s and is a widely used in cancer chemotherapy. However, doxorubicin hydrochloride exhibits a well-recognized cardiac toxicity that is associated with increased cumulative dose and thus limits its clinical use.[2] In an effort to mitigate the well-recognized cardiac toxicity which is associated with increased cumulative doses of doxorubicin hydrochloride, different drug delivery approaches were investigated to improve the selectivity and efficacy of this therapy. These efforts focused on controlled delivery platforms that enabled drug targeting to specific tissues.[3] These efforts led to employing liposomes as drug carriers for chemotherapeutic agents as a potential means to manipulate the drug distribution of the agent and improve anti-tumour efficacy while reducing toxicity.[4] However, it was determined in early studies that liposomes were rapidly recognized and removed from the circulation by the reticulo-endothelial system (RES), which is also known as the mononuclear phagocytic system (MPS), in the liver, spleen and bone marrow.[5,6] Uptake into the MPS not only sequesters the liposomes in the liver, spleen and bone marrow, and clears them from circulation but it is also followed by liposome breakdown and metabolism of the drug in macrophages prior to excretion.[3]	These general comments include a number of issues that are addressed below in relation to 'specific comments'. In addition, while the guidance notes that proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account of quality and non-clinical comparison, where appropriate, the focus of the guideline is on the requirements for pharmacokinetic comparability in line with the remit of the PKWP.

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	Liposomes such as Caelyx® (Doxil® in the United States) are coated with polyethylene (PEG), a synthetic hydrophilic polymer. The bulky PEG headgroup serves as a barrier preventing interactions with plasma opsonins thereby retarding recognition by the MPS and slowing elimination of the liposomes from circulation. These PEG-coated liposomes are referred to as sterically stabilized or STEALTH® liposomes.[7] The STEALTH® technology has resulted in a commercial formulation of pegylated liposomal doxorubicin, known as Doxil® in the United States and Caelyx® in Europe.[3] Since the STEALTH® liposomes remain in circulation longer than unmodified liposomes, the STEALTH® liposomes are able to extravasate from the abnormal vasculature of tumours and accumulate in tumours over the prolonged circulation time, a phenomenon known as the enhanced permeability and retention (EPR) effect.[8] **Public Consultation on the Draft Guidance** As of 5 July 2018, the European Medicines Agency (EMA) requested a public consultation regarding the current draft guidance for assessing the bioequivalence of a potential generic pegylated liposomal doxorubicin drug product to the innovator drug product. In the current draft guidance, the bioequivalence assessment relies on the analysis of liposome encapsulated doxorubicin and unencapsulated doxorubicin (Free Dox) in plasma. Furthermore, the Free Dox concentrations are to be obtained independently and not inferred from the difference between total doxorubicin and encapsulated doxorubicin concentrations. TLC Biopharmaceuticals, Inc. (TLC) proposes a change to the bioequivalence guidance regarding the analytes and the bioequivalence assessment sections of the guidance to better reflect the reality of doxorubicin metabolism and clearance after administration of Caelyx®. TLC proposes that total doxorubicin and encapsulated doxorubicin and its major metabolite doxorubicinol be included as supporting evidence for determining bioequivalence, while removing the Free Dox bioequivalence requirement. Additionally,	

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TLC proposes eliminating the partial AUC requirements from the guidance. The rationale for these changes in analytes and bioequivalence assessment, which is detailed below, is based on the view that efficacy is best represented by the encapsulated doxorubicin concentration and that safety is best represented by the doxorubicinol concentration. Furthermore, TLC suggest that a non-clinical biodistribution analysis also be given weight in support of the bioequivalence evaluation. Therefore, the analyte, additional information and bioequivalence assessment sections of the guidance would appear as shown below, where green highlighted items are additions and red highlighted items in strike-through font are deletions:

Bioequivalence study design	Single dose study: Any dose (but no dose adjustments toxicities during the study) in e.g. stable ovarian/breas cancer patients. Background: Dose proportional pharmacokinetics.
	Cross-over
	Other critical aspects: The single dose study may ne to be conducted with standardized light meals rather th in the fasting state due to patient's needs.
Analyte	☐ total drug ☐ encapsulated drug ~~☐ unencapsulated drug~~ ☐ doxorubicinol (metabolite) ~~**Other critical aspects:** Unencapsulated drug concentrations must be achieved by means of appropri bioanalytical methods rather than by subtracting encapsulated from total drug.~~
	☐ plasma/serum ☐ blood ☐ urine
	Enantioselective analytical method: ☐ yes ☐ n
Bioequivalence assessment	**Main pharmacokinetic variables**: AUC_{0-t}, $AUC_{0-\infty}$, C_m

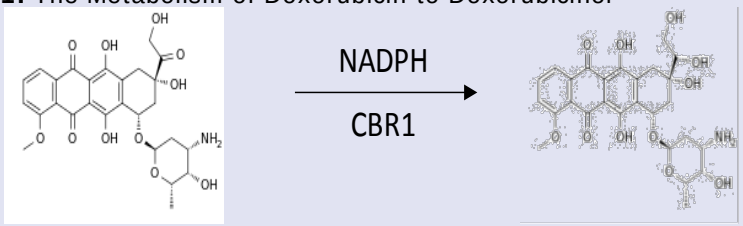
Stakeholder no.	General comment (if any)	Outcome (if applicable)

	~~partial AUCs (e.g. AUC_{0-48h} and $AUC_{48-tlast}$)~~ ~~**Background / justification**: Partial AUCs should ensur profile comparability for the encapsulated compound.~~
	90% confidence interval acceptance limits: 80.00 – 125.00%
Additional information can be added if considered necessary	To be noted: Sponsor is to obtain doxorubicinol bioequivalence (AUC C_{max}, T_{max}), and non-clinical comparison of tissue distribution in animal model(s) between potential drug product and innovator drug product as supporting evidence to the bioequivalence determination. Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator product is considered a step-wise approach which in addition to the pharmacokinetic study also takes accou of quality and non-clinical comparison, where appropria

Appendix I is a representation of the proposed changes to the entire guidance document.

2. Specific comments on text

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
Chapter "Analyte"	1	Comments: The draft Guidance suggests analysing both, encapsulated drug and unencapsulated drug. Proposed change (if any): The necessity to measure the unencapsulated drug in addition to the encapsulated for BE assessment is not supported by the literature. The papers of Gabizon et al., especially Gabizon, A., Shiota, R. and D. Papahadjopoulos: J. Nat. Cancer Inst. 81 (1989) 1484-1488, indicate that PK of unencapsulated doxorubicin (half-life: 20 min) is controlled by the release of the drug from liposomes (half-life: 50 hours). Moreover, the paper of Hsu & Huang (Int. J. Clin. Pharmacol. Ther. 52 (2014) 1071-1082) draws the conclusion from Monte Carlo simulations that "the encapsulated form provides the most accurate assessment of BE for liposome drug products with low reticuloendothelial system uptake" (like doxorubicin). Thus, the encapsulated form was found to be the most sensitive analyte to identify differences between products. In line with the general concept of BE assessment in the EU the encapsulated form should be the primary analyte for BE assessment.	Not accepted. It remains the opinion of the PKWP that both the encapsulated and unencapsulated should be analysed as a basis for pharmacokinetic comparability between two products.
Bioequivalence assessment	1	Comments: Under "Background/justification" it is stated that "partial AUCs should ensure profile comparability for the encapsulated compound". In line with the comment above this statement should include BE assessment based on the encapsulated drug only.	Not accepted. See previous comment also the wording of this background/justification has been amended to make more explicit the requirements.

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): BE assessment should be established on encapsulated doxorubicin used as primary parameter.	
Lines 22-23	2	Comments: **Rational for Inclusion of Doxorubicinol Bioequivalence as Supporting Evidence** Doxorubicinol is the dominant metabolite of doxorubicin and is formed by a two-electron reduction of the ketone moiety on the R_1 group of the anthracycline to an alcohol, depicted in Figure 1. The two-electron doxorubicin metabolism is reduced nicotinamide adenine dinucleotide phosphate (NADPH) dependent, occurs in cellular cytosol and is catalysed by aldo-keto reductase (AKR) and/or carbonyl reductase (CBR1) enzymes.[9] Further exploration of doxorubicin reduction identifies CBR1 as having a higher affinity for doxorubicin than the AKR enzymes, which is significant since CBR1 is also expressed in greater amounts in the human liver than the AKR family of enzymes.[10] Furthermore, CBR1 is localized in the liver in the hepatocytes and Kupffer cells,[11] and is therefore the dominant metabolizing enzyme for producing doxorubicinol in humans.[10] **Figure 1:** The Metabolism of Doxorubicin to Doxorubicinol  In addition, Hilmer et. al. (2004) [12] report that the liposomal form of	Not accepted. Generally, bioequivalence is focussing on formulation differences rather than efficacy of any compound. That is why also prodrugs without pharmacological activity are preferred over active metabolites – if possible – as they would better reflect biopharmaceutic product performance and detect possible formulation differences if they are there. In the case of liposomal doxorubicin the encapsulated drug plus the un-encapsulated compound are considered most relevant for that purpose. Furthermore, because the elimination half-life of doxorubicinol is long, there is a risk of carry-over of doxorubicinol when the second dose is administered.

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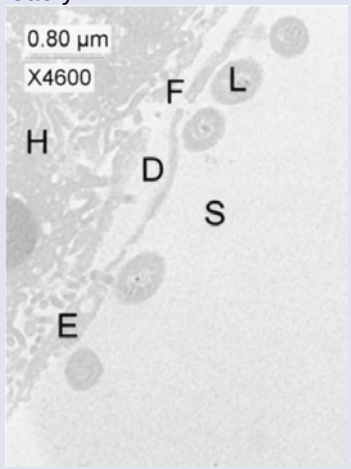
		doxorubicin is not observed in the sinusoidal endothelium, in the hepatocytes or in the Space of Disse employing transmission electron micrographs taken in two hundred ninety (290) rat livers perfused with Caelyx®, as depicted in the representative micrograph in Figure 2. The results indicate that liposomal doxorubicin (innovator drug product, Caelyx®) consisting of ~100 nm mean diameter particles is generally unable to pass through the fenestrations in the sinusoidal endothelium and is restricted to vascular space. Only liposomes with significantly smaller diameters can freely extravasate through sinusoidal fenestrations. **Figure 2:** Transmission Electron Micrograph of Rat Liver Perfused with Caelyx®  A representative transmission electron micrograph of a rat liver perfused with Caelyx®. The liposomes (L) are all seen in the sinusoidal lumen (S). No liposomes were seen crossing the fenestrations (F) in the sinusoidal endothelium	

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		(E), in the Space of Disse (D) or in the hepatocytes (H).[12] There are two primary arguments for using plasma concentration of doxorubicinol rather than of unencapsulated or free doxorubicin (Free Dox) as an assessment of bioequivalence, as follows: 1. <u>The linkage between doxorubicinol in circulation and Free Dox</u>. Although encapsulated doxorubicin is restricted mostly to the sinusoidal lumen, nanoparticles in the sinusoidal lumen are subject to endocytosis by the mononuclear phagocyte system which is in direct contact with the blood streaming through sinusoids.[13] In the liver, Kupffer cells represent the MPS, and pegylation of liposomes substantially decreases and delay their uptake by Kupffer cells and other cells in the liver.[14] The main deposit of liposomal doxorubicin is still the MPS, particularly in the liver, even in the case of pegylated liposomes. Therefore, liver metabolism from Kupffer cells and their neighbour hepatocytes is probably the main source of doxorubicinol in plasma. Because doxorubicinol levels in plasma are consistently about fifty percent (~50%) of those of Free Dox, and the protein binding fraction for both species is about the same at seventy-five to eighty percent (75-80%),[15] measurement of plasma doxorubicinol represents to a large extent a cumulative amount of the Free Dox bio-available in the entire system. Furthermore, the cardiotoxicity associated with doxorubicin therapy is attributed to a large extent to the primary metabolite, doxorubicinol.[15] The doxorubicin associated cardiotoxicity is mitigated in the CBR1 deficient mouse model. The minimization of cardiotoxicity suggests the CBR1 metabolism of doxorubicin to doxorubicinol is the primary cause of the cardiac toxicity associated with doxorubicin therapy.[16] Therefore, the bioequivalence of doxorubicinol is not only an	

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		indirect approximation of Free Dox exposure but also a relevant and more reliable surrogate marker of safety than Free Dox bioequivalence. In addition, the anti-tumour efficacy of the liposomal doxorubicin is mainly rendered by the EPR effect at the tumour site and thus the amount of the locally available doxorubicin as encapsulated doxorubicin and locally-generated Free Dox at the tumour site is much higher than that of the Free Dox in the plasma. Therefore, the bioequivalence of encapsulated doxorubicin in the plasma would be by far more relevant to the anti-tumour efficacy equivalence than the bioequivalence of the plasma Free Dox. 2. Potential *ex vivo* effects on Free Dox concentration in samples. During sample handling and preparation, potential *ex vivo* factors, such as premature breakage of the liposomes during sample handling, may have a confounding effect on apparent Free Dox plasma concentrations and thus will represent additional sources of variation in a sample analysis that is already prone to high variation in a relatively low-level concentration measurement. Although doxorubicinol is also observed as a low concentration analyte, the variability associated with the analysis of doxorubicinol is similar to that of encapsulated doxorubicin and decreased relative to that of Free Dox concentration analysis. As doxorubicinol is only generated *in vivo* through an enzymatic process and is a different molecule from doxorubicin with different high-performance liquid chromatography (HPLC) retention time and different mass spectra, the potential *ex vivo* sources of variation do not affect the variability of the observed concentrations of doxorubicinol.	

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		Proposed change (if any): Therefore, TLC proposes that doxorubicinol plasma concentration should be employed as supporting evidence for bioequivalence evaluation in lieu of Free Dox. Doxorubicinol is a drug product metabolite associated both with anti-tumour efficacy and cardiotoxicity of doxorubicin and thus its equivalence to the innovator product is relevant to the performance of the product. Since doxorubicinol is only formed enzymatically *in vivo*, it is less prone to *ex vivo* sources of confounding and variability from sample handling and preparation in the analyte analysis.	
Lines 22-23	2	Comments: **Rational for Elimination of Unencapsulated Doxorubicin (Free Dox) as a Criteria of Bioequivalence** Encapsulated doxorubicin that accumulates at the tumour site owing to the EPR effect is responsible for the anti-tumour efficacy of treatment. Because of the EPR effect, the amount of the locally available doxorubicin at the tumour site is much higher than that of the Free Dox in the plasma. Therefore, the bioequivalence of Free Dox between potential generic and innovator formulations does not directly correlate to the equivalence of anti-tumour efficacy. Furthermore, encapsulated doxorubicin accounts for greater than ninety-five percent (95%) of the total doxorubicin in circulation [3] and Free Dox is rapidly metabolized to doxorubicinol and other minor metabolites. As noted above, in addition to being formed by hepatic metabolism, doxorubicinol can also be formed in the MPS as CBR1 is available in the macrophages to metabolize doxorubicin to doxorubicinol during the degradation of the liposomes in the macrophage lysosomes. As a result, an intermediary step of liposomal release of Free Dox in circulation to	Not accepted. It remains the opinion of the PKWP that both the encapsulated and unencapsulated drug should be analysed as a basis for pharmacokinetic comparability between two liposomal doxorubicin products.

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		form doxorubicinol is not required and further minimizes the role of Free Dox in assessing the pegylated liposomal drug products bioequivalence. The mechanism of drug leakage of circulating liposomes is not fully understood but includes at least two components: (1) slow leakage from intact circulating liposomes by gradual loss of loading gradient and (2) plasma protein interactions with the phospholipid bilayer causing liposome destabilization and leakage of vesicle contents and phagocytic signalling for rapid removal by the MPS.[17] The two previously mentioned components are minimized in the case of Doxil®/Caelyx® owing to three factors: (1) the ammonium sulphate gradient of the liposomes is very strong and stable in plasma, (2) the drug is mostly in a precipitated gel-phase state forming intra-liposomal rods and (3) the cholesterol-rich, solid bilayer (phase transition temperature is ~52 °C, which is greater than the physiologic temperature of 37 °C), and the surface pegylation contribute to steric stabilization of the liposome membrane preventing the deleterious destabilizing effect of plasma opsonins. In fact, *in vitro* plasma stability assays shave shown that Doxil®/Caelyx® liposomes are extremely stable with minimal drug leakage, as shown in Figure 3.[19] **Figure 3:** The in vitro Stability of Doxil®/Caelyx® Liposomes, (abbreviated as Dox-SL in Figure 3)	

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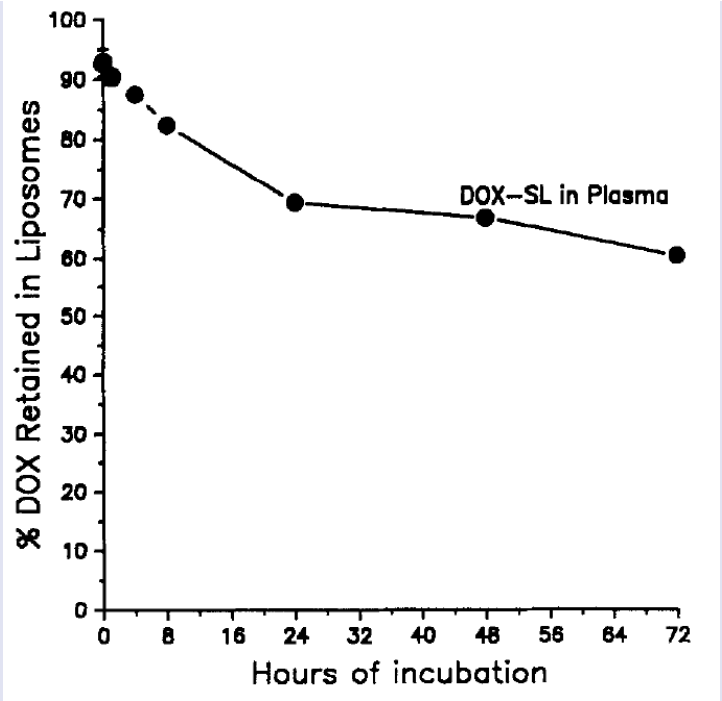

The leakage of drug from DOX-SL after incubation in 90% pooled human plasma. It may be noticed that leakage in plasma is a very slow process ($t_{½}$ * approx. 100 h) and, in principle, may account for only a minor fraction of the liposomal drug clearance in vivo.[19]

Moreover, any free drug that leaks *in vivo* will be rapidly distributed in the body peripheral compartments following the large volume of distribution of Free Dox (~500 L) and/or rapidly excreted in the urine and the bile. It follows that the requirement for Free Dox bioequivalence is actually unjustified and in most cases unfeasible since the analysis is trying to follow

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		a component in plasma that is generated at a lower rate than it is cleared. In addition, sample handling and preparation has the potential to confound the analysis of Free Dox, e.g., alterations of Free Dox levels and encapsulated doxorubicin levels by the premature rupturing of the liposomes. Rupture of liposomes may occur, for example, if samples are inadvertently partially or completely frozen, if they are subject to hypotonic conditions or to other harsh handling procedures. This concern was identified by the scientific advisory working party, which stated that "It is recognized that the extraction procedure could disrupt the liposomes and hence only total doxorubicin may reliably be measured".[20] Further, it has been noted that, given the effect that extraction procedures may on the Free Dox levels and analysis, "Total doxorubicin should be measured, in addition to free and liposome-encapsulated doxorubicin, as an independent verification of the reliability of the separation methodology" as reiterated by both the 2013 reflection paper for generic liposomal products and the scientific advisory working party.[21, 22] A trace amount of prematurely rupturing liposomes would have essentially no effect on the encapsulated doxorubicin analysis as a small change in a high intensity peak is possibly unidentifiable, but for a low intensity peak, like Free Dox, a small change has a potentially significant confounding effect on the accuracy of the analysis. Even an artefactual leakage of 0.1% of the encapsulated doxorubicin during extraction will totally cloud the true values of Free Dox in plasma. The guidance and a confidential communique state the total doxorubicin should be measured for assessing the reliability of the sample separation, but when the total doxorubicin indicates that variance in the Free Dox analysis imply that the separation method has yielded unreliable data, there is no recommended	

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		course of action, for example, not using Free Dox concentrations for bioequivalence determination purposes if the separation method is thought to be unreliable. Consequently, the scientific advisory working party suggested that doxorubicinol should be employed as a surrogate for Free Dox noting, "Nevertheless, doxorubicinol could be determined as a measure of liberated doxorubicin but also as a control for unexpectedly high release."[20] As it appears that both total doxorubicin and doxorubicinol analyses suggest that Free Dox is an unreliable measure of bioequivalence, we believe that bioequivalence should instead be based on the bioequivalence of encapsulated doxorubicin and doxorubicinol, since these parameters directly reflect comparative efficacy and safety, respectively. Despite the fact that direct measurement of Free Dox for a clinical bioequivalence study is a regulatory expectation for liposomal products in general, it poses a technical challenge that not even Janssen Research & Development LLC (Janssen), the innovator of Caelyx®, has yet to achieve. After Janssen voluntarily shutdown the Doxil®/Caelyx® manufacture in Ben Venue Laboratories (BVL) located in Bedford Ohio, USA in 2011, they began plans for manufacturing Doxil/Caelyx at other sites. The site change and approval history are listed on the US FDA's website. As indicated in the approval package of NDA 50-718/S-50,[22] the manufacturing site change application did not include a direct bioanalysis of the Free Dox concentration in its study design, and only added evaluation of Free Dox via a protocol amendment at the US FDA's request using an indirect measure of Free Dox based on the mass balance approach, i.e., the arithmetic difference between total and encapsulated doxorubicin. During the approval package evaluation, the FDA concluded in its executive summary of the clinical	

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		pharmacology review that “An attempt was made to estimate free doxorubicin concentrations as the arithmetic difference between the total and encapsulated doxorubicin plasma concentrations (a mass balance approach), for all subjects and all time points. Because of similarity in the concentrations of total and encapsulated doxorubicin, the differences across the individual time points were small and sometimes negative. These data were not considered suitable for pharmacokinetic analysis or interpretation, other than to note that the low estimated values indicate that only a small fraction of the administered dose is present in plasma as the free (unencapsulated) form”.[22] And in its review comments of Free Dox, the US FDA further conceded that “…the free doxorubicin estimates may not be critical…”[22] Both site change applications were accepted on the basis of bioequivalence on total doxorubicin and encapsulated doxorubicin alone. In addition to this example, published scientific literature also supports that the position that in the specific case of liposomal doxorubicin the concentrations of the total and encapsulated doxorubicin are superior to Free Dox as an indicator for bioequivalence assessment.[23] The PK parameters for total doxorubicin and encapsulated doxorubicin have been employed extensively to assess doxorubicin levels *in vivo*. [24, 25] Historically, the concentration of Free Dox has been determined by the difference of total doxorubicin and encapsulated doxorubicin concentrations. This is owed to the low concentration of Free Dox, the difficulty in obtaining Free Dox without total and/or encapsulated doxorubicin “contamination”, and analytical methods robust enough to accurately and precisely measure the Free Dox in an *in vivo* matrix.	

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		Finally, there is significant intra- and inter-individual variability in measuring Free Dox in patients. The intra- and inter-individual variabilities have been estimated at 6-59% and 37-93%, respectively.[26] Although, a crossover study should help mitigate inter-individual variability by having an individual accounted for in both arms of the study, the significant intra-individual variability associated with Free Dox may reduce this effect as an individual patient may not mirror themselves from one arm of the study to the next. The inter-individual variability for Free Dox has been cited for the PK parameters of $AUC_{0-\infty}$, total clearance and terminal $t_{1/2}$.[26] Likewise, the intra-individual variability for Free Dox has been cited for the PK parameters of $AUC_{0-\infty}$, total clearance and terminal t $_{1/2}$.[27] The irregularities in the PK parameters are not attributed to cumulative dose but introduce a higher degree of variability in the Free Dox concentration measurement that cannot be mitigated by the crossover study design of the bioequivalence study. Proposed change (if any): For these reasons, TLC proposes that doxorubicinol plasma concentration be utilized in lieu of Free Dox as an analyte for bioequivalence evaluation to demonstrate bioequivalence. Since Free Dox is not associated with product efficacy or safety, TLC suggest employing Free Dox for descriptive purposes only.	
22-23	2	Comments: **Specification of the Guidance on Partial AUC Requirements** In the current form of the guidance, the partial AUC requirement is stated as, “Partial AUCs should ensure profile comparability for the encapsulated compound.” The wording of this guidance statement reads that the partial AUC requirement is solely for the encapsulated doxorubicin analyte. If so,	Partly accepted. The wording of the background/justification has been amended to read *AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for encapsulated and unencapsulated drug. Partial AUCs for the encapsulated drug to ensure profile comparability*.

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		please add a statement that the partial AUC is not required for the unencapsulated analytes. Proposed change (if any): Based on possible significantly difference in interpretations of this requirement, TLC suggest that EMA add a statement that the partial AUC is not required for the unencapsulated analytes.	
22-23	2	Comments: **Rationale for Elimination of Partial AUCs** The topic of partial AUC has been discussed on numerous occasions at national and international meetings and mentioned in the 2013 Reflection paper. The cut-off point selection has been debated for a long time but in this guidance, draft was codified as partial AUC_{0-48h} and partial $AUC_{48h-\infty}$. This selection of cut-off point at 48 hours does not appear to correlate with any significant factor identified in the full AUC profile, AUC_{0-t} or $AUC_{0-\infty}$. The US FDA recommends that the time at which partial AUC is truncated should be related to clinically relevant pharmacodynamics (PD) measurement, and a sufficiently quantifiable sample be collected to allow adequate estimation of the partial AUC.[28] However, as Caelyx® for cancer treatment requires multiple cycles of administration to exhibit its anti-tumour efficacy, it is not possible to identify a clinically relevant PD measurement within the measurable PK time span under the proposed bioequivalence study design. Therefore, there is no apparently meaningful cut-off point that can be selected for partial AUC bioequivalence evaluation between Caelyx® and its generic candidates.	Not accepted. It remains the PKWP opinion that partial AUCs are required for the encapsulated drug to ensure profile shape comparability. Of note, this position is supported by current research findings as presented at the EUFEPS 3rd Global Bioequivalence Harmonisation Initiative conference (conference report awaited to be published) held in Amsterdam April 2018.

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		Comparison of the mode of action of IR/DR drugs and liposomal doxorubicin hydrochloride and its effect on the relevance of partial AUC as an indicator of equivalence. Partial AUC is area under the curve segments determined between given time points, including early partial AUCs and terminal partial AUCs. The U.S. FDA recommends the use of partial AUC as an early exposure measure under certain circumstances in their draft guidance "Bioequivalence Studies with Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA". As an example, one such special circumstance would be when rapid onset is critical to the safety and/or efficacy of the drug product, such as DOCK No. FDA-2005-0120 regarding Adderall (methylphenidate) XR, whose formulation is 1:1 ratio of immediate release (IR) and delayed release (DR) pellets in a gelatine capsule that is designed to achieve both rapid onset of activity and sustained activity through the day.[28] Similarly, Health Canada recommends using partial AUC when the early exposure of an immediate release drug product is important.[29] In contrast, the efficacy of the pegylated liposomal formulation of doxorubicin hydrochloride is drastically different not dependent upon early onset and has no immediate release component, distinguishing it from the kinetic-wise IR/DR formulations. Possibly for this reason, the FDA draft guidance on doxorubicin hydrochloride does not require partial AUC as a demonstration of the bioequivalence of generic and innovator formulations of pegylated liposome doxorubicin hydrochloride.[30] The encapsulation ratio of Caelyx® is set at NLT 98%, which means that there is less than 2% free form doxorubicin (Free Dox) in the formulation.	

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		This amount of Free Dox does not contribute significantly to the efficacy because it is a minimal dose of ≤1 mg/m^2, i.e., at least 20-fold below the lowest dose of doxorubicin (20 mg/m^2) that has shown some efficacy in weekly-dosing protocols. The anti-tumour activity of the pegylated liposomes is mostly contributed by the impact of the EPR effect on the biodistribution of the liposomes. After drug administration, ratios of free to encapsulated doxorubicin concentration at each sampling time-points were under 2%, and the drug is mainly represented as the encapsulated form in the circulation (>95%). There is a clear difference between the mechanism of action of classical IR/DR formulations and liposomal doxorubicin hydrochloride, in which the IR fraction (which would equate to the initial Free Dox in the formulation) is considerably below the pharmacological threshold of activity, indicating that partial AUC is not a relevant indicator of bioequivalence for the latter and that is should not be included in EMA bioequivalence guidance for pegylated liposomal doxorubicin hydrochloride. General disadvantages of relying upon partial AUCs. Other more general disadvantages of using partial AUCs as a requirement for bioequivalence have been reported. The primary disadvantage is that partial AUC may be extremely heterogeneous compared to the more robust full AUC. As reported by Walter, an empirical truncation process complicates the interpretation of partial AUC and makes the assessment problematic. One such problematic issue is that the partial area lacks a useful symmetry property enjoyed by the full AUC. By dividing the full AUC into parts, undue scrutiny and influence may be given to a partial AUC that may have little to	

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		no relevance to the overall action of the object of study.[31] DiLiberti has discussed other performance issues with partial AUCs, including that they may prone to high within-subject variability, they may be overly discriminating (e.g., causing different lots of originator product to be declared inequivalent), they may be significantly subject to the undue influence at clinically insignificant regions of PK curve (where drug concentrations are too low to have meaningful efficacy) and, finally, that small changes in the time course of the PK profile can have inordinately large effects on partial AUCs, etc.[32] Statistical considerations in using partial AUCs. Finally, the use of partial AUC increases the Type II error rate in evaluating bioequivalence. A retrospective study of prolonged release formulations was conducted to determine which would have failed equivalence under the proposed EMA partial AUC parameters. Twenty percent (20%) of single dose and forty percent (40%) of paired fed/fasting products that were equivalent with full AUC parameters would fail the partial AUC parameter. A chief cause was high variability in the assessed parameter.[33] Proposed change (if any): Based on above reasons, we believe that partial AUC as a bioequivalence assessment criterion of liposome doxorubicin hydrochloride study is not relevant and may, in fact, be inaccurate. Thus, we proposed that partial AUCs be provided descriptively for information purposes only.	
Line 22-23	2	Comments: **Rationale for Elevation of the Evaluation of Non-clinical Doxorubicin**	Not accepted. While the guidance notes that proving equivalent efficacy and safety of a

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		and Doxorubicinol Biodistribution as Supportive Evidence As stated in the 2013 reflection paper for generic liposomal products, "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".[34] It was further stated that "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".[34] Encapsulated doxorubicin accumulated in the tumour is responsible for the efficacy of the treatment. Likewise, accumulation of doxorubicin and doxorubicinol in the heart is responsible for the cardiac toxicity associated with the therapy. As communicated by scientific advisory working party, "The safety and efficacy of liposomal doxorubicin is influenced by its tissue distribution and subtle changes in the formulation have been shown to alter its pharmacological activity and toxicity [Roa, et al, Cancer Chemother. Pharmacol., 66: 1173-1184 (2010)]".[21] Not only should the biodistribution be comparable between the innovator drug product and the potential generic drug product, but according to the scientific advisory working party "Minimally, the Applicant should demonstrate comparable drug distribution to the liver, spleen, kidney and tumour but also to the skin and heart given the propensity for skin toxicity and cardiotoxicity for this compound class".[21] Therefore, the similarity between the innovator and the potential generic	liposomal formulation developed to be similar to an innovator product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account of quality and non-clinical comparison, where appropriate, the focus of the guideline is on the requirements for pharmacokinetic comparability in line with the remit of the PKWP. As a general note, non-clinical biodistribution studies are considered unsuitable as "optional supportive aspect for bioequivalence evaluation" as proposed by TLS because there are no criteria how to handle that. If bioequivalence study outcome is successful, there is no need for additional data and if the study is not demonstrating bioequivalence in humans, this cannot reasonably be covered by experimental non-human data.

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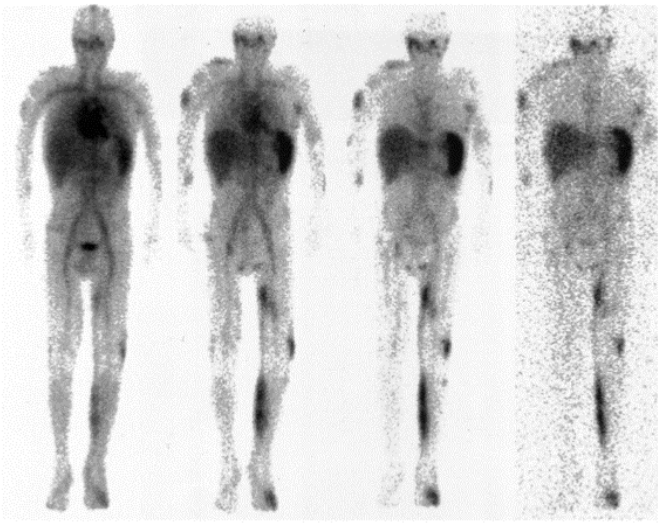
		drug product of doxorubicin and doxorubicinol organ distribution also estimates the bioequivalence of the potential drug product. A non-human study to assess doxorubicin and doxorubicinol distribution after administration of the liposomal drug products, since such studies are not feasible in humans, allows quantification of the tissue distribution of doxorubicin and doxorubicinol. Although not quantitative, a limited number of gamma scintigraphic studies of patents administered radio-labelled pegylated liposomal doxorubicin demonstrate that using pegylated liposomes carrying a radio-opaque marker showed that the liposomes preferentially accumulated in tumours, as shown in the example in Figure 4.[35, 36] **Figure 4:** Series of Scintigraphs of Radiolabeled Liposomes	

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome



Serial whole-body gamma camera images over seven (7) days of a patient with stage $T_1I_1S_1$ AIDS-KS. Multiple areas of uptake of radiolabeled liposomes are seen in the left foot and leg, right arm, and face. Each of these areas corresponded with a typical Kaposi sarcoma lesion. Prolonged retention of the radiolabel is seen despite significant clearance of circulating liposomes, as demonstrated by disappearance of the cardiac blood pool image.[35, 36]

As depicted in Figure 4, the encapsulated radiolabel preferentially accumulated in the tumour regions as early as 4-hours post administration and is retained in the tumour regions once circulating liposomes have been significantly cleared, after 72-hours post administration. These studies were done in patients with Kaposi sarcoma tumours, and studies in animals have shown accumulation in other types of tumours as well. These studies

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		support the claim that providing comparative biodistribution information on a potential generic drug product and the innovator product help to establish the overall bioequivalence. Proposed change (if any): Although the 2013 reflection paper for generic liposomal products states, "There is insufficient regulatory experience of such studies to support specific decision criteria for comparability of tissue distribution",[34] a non-clinical biodistribution study assessing the tissue distribution of doxorubicin and doxorubicinol between the innovator and potential generic drug product would assist the assessment of the bioequivalence determination of the circulating analyte concentrations of encapsulated doxorubicin and doxorubicinol. The biodistribution study should be considered an optional supportive aspect in the equivalence evaluation of the potential drug product. **REFERENCES** 1. Thorn, C.; Oshiro, C.; Marsh, S.; Hernandez-Boussard, T. McLeod, H.; Klein, T. and Altman, R., "Doxorubicin pathways: pharmacodynamics and adverse effects", **2011**, *Pharmacogenet. Genomics*., 21(7): 440-446. 2. Theodoulou, M. and Hudis, C., "Cardiac Profiles of Liposomal Anthracyclines Greater Cardiac Safety versus Conventional Doxorubicin?", **2004**, *Cancer*, 100(10): 2052-2063.	

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
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Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
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Adopted by CHMP for release for consultation	31 May 2018
Start of public consultation	5 July 2018
End of consultation (deadline for comments)	30 September 2018

Comments should be provided using this template. The completed comments form should be sent to PKWPsecretariat@ema.europa.eu

Keywords: Bioequivalence, generics, pegylated liposomal doxorubicin hydrochloride

Disclaimer: *This guidance should not be understood as being legally enforceable and is without prejudice to the need to ensure that the data submitted in support of a marketing authorisation application complies with the appropriate scientific, regulatory and legal requirements.*

Requirements for Bioequivalence Demonstration (PKWP)*

Bioequivalence study design	Single dose study: Any dose (but no dose adjustments for toxicities during the study) in e.g. stable ovarian/breast cancer patients. Background: Dose proportional pharmacokinetics.
	Cross-over
	Other critical aspects: The single dose study may need to be conducted with standardized light meals rather than in the fasting state due to patient's needs.
Analyte	● **total drug** ● **encapsulated drug** ~~○ unencapsulated drug~~ ❍ **doxorubicinol (metabolite)** **Other critical aspects:** ~~Unencapsulated drug concentrations must be achieved by means of appropriate bioanalytical methods rather than by subtracting encapsulated from total drug~~**.**

Line no.	Stake-holder no.	Comment and rationale; proposed changes	Outcome
		● plasma/serum ❍ blood ❍ urine Enantioselective analytical method: ❍ yes ● no	
		Bioequivalence assessment	**Main pharmacokinetic variables**: AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, ~~partial AUCs (e.g. AUC_{0-48h} and $AUC_{48-tlast}$)~~ **Background / justification**: ~~Partial AUCs should ensure profile comparability for the encapsulated compound.~~
			90% confidence interval acceptance limits: 80.00 – 125.00%
		Additional information can be added if considered necessary	To be noted: Sponsor is to obtain doxorubicinol bioequivalence (AUC_{0-t}, C_{max}, T_{max}), and non-clinical comparison of tissue distribution in animal model(s) between potential drug product and innovator drug product as supporting evidence to the bioequivalence determination. Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator product is considered a step-wise approach which in addition to the pharmacokinetic study also takes account o quality and non-clinical comparison, where appropriate.

* As intra-subject variability of the reference product has not been reviewed to elaborate this product-specific bioequivalence guideline, it is not possible to recommend at this stage the use of a replicate design to demonstrate high intra-subject variability and widen the acceptance range of C_{max}, C_τ, ss and partial AUC. If high intra-individual variability (CV_{intra} > 30 %) is expected, the applicants might follow respective guideline recommendations.

Item – green highlighted items are additions to the guidance
Item – red highlighted items are deletions from the guidance