26 March 2015
EMA/674663/2014
Committee for Human Medicinal Products (CHMP)

Overview of comments received on 'Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin and insulin analogues' (EMEA/CHMP/BMWP/32775/2005_Rev.1)

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

Stakeholder no.	Name of organisation or individual
1	European Biopharmaceutical Enterprises (EBE)
2	European Biosimilars Group (EBG), a sector group of the European Generic medicines Association (EGA)
3	Wockhardt Ltd.
4	Medicines Evaluation Board (MEB, NL)

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1. General comments – overview

Stakeholder no.	General comment (if any)	Outcome (if applicable)
1	EBE welcomes the opportunity to discuss the second draft of the revised guideline on nonclinical and clinical development of similar biological medicinal products containing recombinant human insulin. It is especially important that insulin analogues and long acting human insulin preparations are included in the revised guideline. We note with appreciation that the new draft incorporates many of the comments given by EBE in the previous round. The activities to establish comparability between proposed biosimilar insulin and the originator depend on the mode of action of the insulin as well as on other clinically relevant properties. It is therefore important that the guideline reflects the EMA's view on the different types of insulins.	Acknowledged.
	Today there are basal long-acting insulins with a duration of action that is longer than the intended dosing frequency. For these compounds, single dose studies or studies that do not establish steady state are not suitable for evaluating similarity in human PK/PD. Instead, multiple dose studies measuring PK/PD at steady state are recommended. For safety reasons, as well as for securing a proper read-out, such studies should not be made in healthy volunteers, but can be performed in patients with T1DM.	No change to the guideline warranted. The guideline highlights the relevant issues related to clamp studies for the purpose of comparing long-acting insulins. However, it is recognized that such studies have been successfully performed in both patients with T1DM or in healthy volunteers and have been used to compare the PK and PD profiles of insulins (including long-acting insulins) in the context of comparability exercises required for changes in the manufacturing process or for a biosimilar development.
	While the pharmacodynamic specifics of long-acting insulin preparations are discussed (lines 290-309) in this guideline, we are	No change to the guideline warranted. The guideline already includes in the PD endpoint section

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	missing the specifics for rapid acting insulins. This should be added. Especially, for rapid acting insulins it is not the duration of action but the onset of action, which is the essential PD-parameter for which comparability should be requested.	the following sentence: "Other meaningful pharmacodynamic endpoints are time to onset of action and tGIRmax for rapid-, short- and intermediate-acting insulins and partial GIRAUC (such that are meaningful for the respective insulin)."
1	The level of details provided in this guidance about the clamp procedure has been adjusted since the last draft but the guideline should stay high level. The statement that the analyte concentration should reflect the exogenous insulin without or with negligible interference from endogenous insulin should suffice and the applicant should ensure that the dynamics are reflective of the exogenous insulin.	Not accepted. Detailed guidance on this issue has been requested previously and it should be kept in mind that this guideline does not only provide guidance for developers of biosimilar insulins but also to assessors reviewing the respective applications.
2	EBG welcomes this second round of revision of the 'Guideline on non-clinical and clinical development of similar biological medicinal products containing recombinant human insulin' including the requirements for insulin analogues. Several changes proposed by EBG were implemented in the draft second revision in line with the progressive approach of the CHMP with respect to the development of similar biological medicinal products (i.e. biosimilars). It needs to be appreciated that the non-clinical development as provided and confirmed in the current draft second revision is viewed as an integral part of current efforts to reduce animal testing deemed to be unnecessary based on scientific background. As a general comment on the first draft revision, EBG noted that the clinical section of the draft revision was remarkably detailed as compared to the relative concision of other product specific guidelines	Acknowledged. Acknowledged. Acknowledged.

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	issued by CHMP up to now. This statement particularly was true for the section on pharmacology studies where the methodological and practical aspects of the clamp studies are discussed in abundant detail. Now it has to be acknowledged that the CHMP considerably reworded this section and the details previously provided in the clinical pharmacology study section have been reduced preserving the flexibility in order to allow for equally appropriate solutions. EBG suggests keeping the clinical pharmacology sections in the current form.	
	Regarding clinical efficacy, EBG fully agrees with the statement in the corresponding section that apart from the comparative pharmacokinetic and pharmacodynamic assessment there is no anticipated need for separate efficacy studies.	Acknowledged.
	On the first revision of this guideline, EBG noted that the requirement for a long term safety study to assess comparative immunogenicity is deemed to be excessive without justification. EBG welcomes that in the second revision this comment was given attention by the CHMP. Firstly, the duration of the safety study has been determined as appropriately justified, 6 month comparative safety data as a maximum. Secondly, even more importantly, as is stated in the second revision, in certain cases, a pre-licensing safety study including immunogenicity assessment may be waived given that high level of comparability has been demonstrated in the quality, in vitro and PK/PD profiles. EBG strongly suggests keeping the current flexible wording of CHMP's progressive approach.	Acknowledged.
	Additionally, it has to be appreciated that many specific comments made by EBG on the first revision of the text have been taken into	

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	account and implemented by the CHMP in the second revision. Therefore EBG recommends keeping these modifications in the final version.	
3	As per the current EMA's Q&A document ('EMA Procedural advice for users of the Centralised Procedure for Similar Biological Medicinal Products applications', EMA/940451/2011), the European Commission has confirmed that it intends to accept batches of reference (approved) biological products sourced from outside the EEA in certain pre-clinical and clinical studies for the comparability exercise. Under this approach, representativeness of the batches sourced outside the EEA with those authorised in the EEA will be mainly achieved through an "extensive analytical comparison" (*in vitro* CMC comparisons). However, for some cases "comparative PK and PD data" may be required. Considering that: - Insulins are generally very well characterised protein medicinal products, widely used since decades - No *in-vivo* PK/PD studies are generally required for biosimilar insulin comparability exercise (see lines 123 to 126 of the current draft), we propose to broadly consider insulin development as a specific case (with reference to the overarching biosimilar guideline) and clearly specify that for establishment of the representativeness of the reference insulin batches sourced outside the EEA with those authorised in the EEA, no comparative PK and PD data between those batches are expected as a rule. For establishment of such representativeness, extensive analytical comparison (*in vitro* CMC comparisons) should be considered sufficient.	Not accepted. Although insulins can be well characterised, differences between the EEA-sourced and non EEA-sourced original product, e.g. in formulation, may affect the PK profile and may necessitate comparative PK studies. The Applicant will need to justify that the analytical comparison is sufficient.
4	The guideline is well-written and provides adequate guidance on the	Regarding the need for mitogenicity testing the arguments

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	development of biosimilar insulines and the requirements for marketing applications. We support that the guideline now clearly expresses the view that biosimilar comparability studies should be done at the level and with the assays which are most sensitive in picking up any differences. Despite this general support of the guideline we have some minor comments as detailed below. With respect to the nonclinical studies the MEB supports the emphasis on the in vitro studies, and considers in vivo animal studies not necessary at all. With respect to the in vitro endpoints the MEB suggests to focus (in addition to receptor binding) more on pharmacodynamic properties, i.e. on metabolic activity only. We admit that the overarching Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues (draft revised text), indicates: "Together, these assays should cover the whole spectrum of pharmacological/toxicological aspects known to be of clinical relevance for the reference product and for the product. However, increased mitogenic activity is mainly a property of insulin glargine, and less prominent for other insulins. Even for insulin glargine this mitogenic property is hardly clinically relevant in in vivo situation. Mitogenic properties will in general, therefore, not add to the evaluation of biosimilarity. The paper of Ter Braak[1] et al, 2014, is referred to support these changes.	by the MEB are accepted and the guideline has been changed as follows. "In general, mitogenic activity mediated by IGF-1 receptor stimulation might not be relevant for human insulin and for most insulin analogues. However, if applicable, comparative IGF-1 receptor binding and an assay for functional activity can be included to cover this potential toxicological effect."
4		

[1] Ter Braak B., Siezen CLE, Kannegieter N, Koedoot E, Van de Water B, Van der Laan JW. 2014 Classifying the adverse mitogenic mode of action of insulin analogues using a novel mechanism-based genetically engineered human breast cancer cell panel. Arch. Toxicol. DOI 10.1007/s00204-014-1201-2

2. Specific comments on text

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
41-42	1	Comment: Insulin analogues are structurally modified derivatives of human insulin (substitutions of amino-acids or other chemical changes within the human insulin) that may confer altered metabolic and/or mitogenic potency, which should be addressed by biosimilar sponsors. Proposed change (if any): "Insulin analogues differ from human insulin by the substitution of amino acids or other chemical changes such as addition of a fatty acid chain within the molecule. These changes are normally incorporated to alter PK/PD properties and may confer altered metabolic and/or mitogenic potency".	Not accepted. For the purpose of the guideline, there is no need to include the notion that structural modifications may alter mitogenic potency. Human insulin and insulin analogues can be well characterised and there is no anticipated need to generally investigate mitogenicity of the biosimilar. (see comment above).
47-48	1	Comment: Co-formulations with insulin are also entering the market and should be mentioned. Proposed change (if any): "…and are used alone or as free mixtures, or premixed preparations of rapid/short-acting insulin and intermediate/long-acting (biphasic) insulin in various proportions or in co-formulations with other diabetes drugs."	Partly accepted. Mix insulins are already mentioned in the guideline. No further specific guidance is considered necessary.
49	1	Comment: As it is not possible to completely rule out differences in higher order structures the word	Not accepted. The current wording is considered appropriate. Insulins are

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		"comprehensively" seems to convey too high expectations. One of the following two alternatives should be considered: Proposed change (if any): "…methods are available to comprehensively characterize in detail the primary, secondary and tertiary structures of the recombinant insulin molecule…" or "…methods are available to comprehensively characterize the primary structure, and to characterize in detail the secondary and tertiary structures of the recombinant insulin molecule…"	well characterisable molecules with state-of-the-art methods.
51-54	1	Comment: Alternative expression cell systems are now being used in the production of insulin analogues, some of which may carry an increased risk of undesirable post-translation modifications, such as glycosylation. Glycosylated forms should also be acknowledged as a process related impurity in need of extra attention. Proposed change (if any): Attention should be given to product related substances/impurities and process related impurities, and in particular to desamido forms, glycosylated forms and other forms that may derive from the expression vector or arise from the conversion steps removing the C-53 peptide and regenerating the three-dimensional structure.	Not accepted. No need to specifically mention glycosylated forms. This is covered by the term "impurities".

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53	4	Comment: In line the words 'expression vector' are used. It is not entirely clear what is meant here. 'host cell' or 'vector construct'. Proposed change (if any): Please clarify, e.g. use the more encompassing words 'expression system'	Paragraph has been reworded.
72-90	1	Comment: In the list of relevant guidelines, it would be appropriate to also mention the "Points to consider document on the non-clinical assessment of the carcinogenic potential of insulin analogues". Insulin analogues have specific characteristics such that, in the assessment of carcinogenic potential, the evaluation of the total activity profile of the compound in vitro and in vivo must be considered relevant. Proposed change (if any): Add in section 3:"Points to consider document on the non-clinical assessment of the carcinogenic potential of insulin analogues (CPMP/SWP/372/01)".	Partly accepted. Human insulin and insulin analogues can be well characterised and there is no anticipated need to generally investigate mitogenicity/ carcinogenicity of the biosimilar. The wording has been modified to read: "In general, mitogenic activity mediated by IGF-1 receptor stimulation might not be relevant for human insulin and for most insulin analogues. However, if applicable, comparative IGF-1 receptor binding and an assay for functional activity can be included to cover this potential toxicological effect."
109-110	1	Comment: We agree that comparative receptor binding should be shown for human insulin as well as human IGF-1 receptors. However, we do not consider that determination of on-off kinetics is necessary for the following reasons: 1) The receptor-ligand rate constants k (on) and k (off) are related to the equilibrium dissociation	Not accepted. The granularity of information is decreased when only dissociation constant is measured. The same requirement is in place for other biosimilars.

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		constant (Kd) by the formula Kd = k (off)/k(on). Thus, differences in k (on) or k (off) are normally detected when assessing the receptor-ligand affinity under equilibrium conditions using the standard competition binding assay. Only in the rather unlikely case that k(on) and k(off) of the insulin biosimilar are changed to the same extent, similar affinity constants would be measured despite differences in k(on) and k(off). 2) As changes in k (on) and k (off) are correlated with shortened or prolonged receptor activation, this should be detected by the recommended functional assays addressing the biological activity of the insulin biosimilar, i.e. receptor autophosphorylation of human insulin and human IGF-1 receptor, metabolic activity and mitogenic activity, even in the case of similar ligand affinities. Proposed change (if any): Comparative receptor binding, including on-off kinetics should be shown for human insulin as well as human IGF-1 receptors.	
109-110	4	Comment: The Insulin receptor consists of two types, IR-A and IR-B, both are involved in the metabolic activity. There is insufficient reason to include human IGF-1 for a biosimilar, as it is not the main target of activity. Proposed change (if any): Comparative receptor binding on both IR-A and IR-B receptors, including on-	Accepted.

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		off kinetics should be shown for human insulin as well as human IGF-1 receptors.	
111-112	4	Comment: The mitogenic activity should not be an endpoint for biosimilarity for all insulins. Proposed change (if any): Biological activity should be compared primarily on pharmacodynamic properties at three two levels: receptor autophosphorylation, and metabolic activity and mitogenic activity. In general, mitogenic activity mediated by IGF-1 receptor stimulation might not be relevant for most insulin analogues. However, if applicable, comparative IGF-1 receptor binding and an assay for functional activity can be added to cover this potential toxicological effect.	Accepted.
114-118	4	Comment: It might be important to have a confirmation from different in vitro endpoints, to avoid that a certain assay might lead to an error. Proposed change (if any): For metabolic endpoints various assays are available, including glycogen formation, lipogenesis, inhibition of stimulated lipolysis as well as glucose transport, which can be studied in a variety of cells. There is no need to do them all; There is a need to include at least three different assays to have internal confirmation. Any of such assays may suffice as long as the data provide a clear view on how insulin receptor agonistic properties of biosimilar and	Accepted.

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		reference product compare.	
118-121	4	Comment: The sentence regarding the functional activity on the IGF-1 receptor can be dropped. Proposed change (if any): Functional activity of the IGF-1 receptor can be evaluated by testing mitogenic potential in cells expressing this receptor. For all endpoints (receptor autophosphorylation, and metabolic effects and mitogenicity), different experimental approaches exist.	See comment above.
125	4	Comment: In vivo studies are not required. This might be stated more explicitly. Proposed change (if any): and are normally not required as part of the comparability exercise.	Accepted
136-138	4	Comment: Referring to the mitogenic activity is no longer needed. This avoids also an association that for a biosimilar the need for assessment of carcinogenic potential would still be important. Proposed change (if any): Although measuring mitogenic activity (in vitro) is expected for comparison of functional activity of biosimilar and reference product, there is no need to perform carcinogenicity studies.	Accepted
143-145	1	Comment: Single dose studies or studies that do not	Not agreed.

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		establish steady state are not appropriate for evaluating insulins with a duration of action that is longer than the intended dosing frequency. Single-dose data will be misleading and may, in fact, give the wrong interpretation. It is, for example, not possible to evaluate the duration of action and clinical relevant exposure/effect level for some long-acting insulins from single dose studies. Evaluation of PK and PD properties of long-acting basal insulins should instead be performed after multiple doses using the intended dosing frequency (e.g. once daily, once weekly) and at steady state. Proposed change (if any): “For this purpose, cross-over, preferably double-blind insulin clamp studies using single or multiple subcutaneous doses (depending on whether rapid/short or long-acting insulin preparation are being investigated) of the test and reference agents are considered most suitable.	Clamp studies as described in the guideline have been successfully employed to compare time-concentration and time-action profiles also for long-acting insulins.
156-158	1	Comment: It is unclear what the implication or recommendations are based on the statement on differences in endogenous insulin levels in different ethnicities. As the recommendation is to perform comparative crossover PK/PD studies, where each subject is their own control, ethnic difference in endogenous insulin levels (if there are indeed such differences) should not matter. As stated in lines 150-152, a homogenous population should be used in order to allow differences in formulations to be displayed.	Accepted.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		Proposed change (if any): Consider deletion.	
166-168	1	Comment: Evaluation of PK and PD properties of long-acting basal insulins should be performed after multiple doses and at steady state. For safety reasons, it is not considered feasible to use healthy subjects for multiple dosing studies using clinical relevant doses (>0.3 U/kg). Addition of text suggested for increased clarity. Proposed change (if any): Clamp studies including either healthy subjects or patients with T1DM are considered appropriate for comparison of insulins with a short or intermediate duration of action, while patients with T1DM are preferable for comparison of long-acting insulins. For long-acting insulins where multiple dose studies at clinically relevant doses (≥0.3 U/kg) are preferable it is not considered feasible, for safety reasons, to use healthy subjects.	Not accepted. See comment above.
176	1	Comment: Clarification is requested on the statement "Measurements of plasma insulin concentrations and glucose infusion rate (GIR) allow an estimation of the time-concentration and time-action profile, respectively, and, if investigated in the same clamp study, of the relation between exposure and glucose-lowering effect." Is it intended as a requirement to estimate the relation between exposure and glucose-lowering effect?	Accepted. There is no requirement to formally compare the relationship between exposure and glucose-lowering effect. The whole sentence has been deleted to avoid confusion and to reduce redundancies.

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201-204	1	Comment: The guideline states: “For evaluation of prandial insulins, the insulin bolus is expected to largely suppress endogenous insulin for the duration of the clamp.” This seems too vague. Evidence of the levels of endogenous insulin (or subtraction, especially if the assay is not specific for the test/reference products) should be required. Proposed change (if any): “For evaluation of prandial insulins, the insulin bolus is expected to largely suppress endogenous insulin for the duration of the clamp. Endogenous insulin can usually be sufficiently suppressed by clamping blood glucose levels below the subject’s fasting glucose (see below). Evidence of the levels of endogenous insulin should be generated. If the assay is not specific for the test/reference product, subtraction may be used.	Not accepted. The current wording is considered to provide clearer advice. However, the paragraph has been slightly modified to further improve clarity.
211	1	Comment: It is not clear if an actual correction of endogenous insulin is expected. The guideline only requires that C-peptide should be measured. To ensure that the extent and consistency of suppression is adequate, evidence of the levels of endogenous insulin (or subtraction, especially if the assay is not specific for the test/reference products) should be required (see comment above).	Measurement of serum C-peptide but not insulin is useful to estimate the extent and consistency of suppression of endogenous insulin. In the absence of insulin suppression, C-peptide correction methods may be considered but this is not a requirement since the value of these methods is not established. Therefore, the guideline states that, regardless which method is used, it should be justified and consistent throughout the clamp studies to ensure comparable test conditions.

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217	1	Comment: Doses for demonstration of equivalence should be in the order of therapeutic doses. In the rare event of concentrations being too low for robust bioanalytical assessments, higher supra-therapeutic doses are acceptable. For insulin products requiring accumulation of exposure to achieve therapeutic concentrations, demonstrating equivalence in steady state conditions may be appropriate. For example, sustained release formulations such as long- to ultra-long acting insulin products with apparent half-lives > 12 hours, are based on accumulation of exposure. Proposed change (if any): “For insulin products requiring accumulation of exposure to achieve therapeutic concentrations, demonstrating equivalence in steady state conditions may be appropriate.”	Not accepted. The doses recommended in the guideline are within the therapeutic range and experience shows that they elicit a robust PD response. Regarding steady state PK/PD studies, see comment above.
242-260	4	tmax/ cmax could be added as secondary or even tertiary parameter for long-acting insulins; major deviations together with uncertainties on the quality part might imply information about the formulation	Partly accepted. The guideline now clarifies that for long-acting insulins, in some cases, determination of Cmax and tmax may not be possible and may be clinically meaningless. In such cases determination as secondary endpoints also does not make sense.
243	1	Comment: Non-specific assays do not provide equally accurate and reliable PK data as specific assays, especially if healthy subject are used. As mentioned in the Study Population section of this guideline, specific assays capable of distinguishing between exogenous and endogenous insulin are not available for all	Accepted. This aspect has been included in the guideline.

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		insulins. However, for the analogues where specific assays do exist, or can be developed, use of a specific assay should be required in the evaluation of PK properties. In general, the assay validation and the bioanalytical report must be available for documentation for the validity of the pharmacokinetic results. Proposed change (if any): Where possible, specific assays capable of distinguishing between exogenous and endogenous insulin should be used to evaluate PK properties.	
250-252	1	Comment: It is mentioned that "AUC(0-τ) should be the primary endpoint and measures of partial AUCs, e.g. AUC(0-τ50%) and AUC(τ50%- τ), the secondary endpoints. T1/2 should be determined where possible." AUC(0-τ) is the AUC at a dosing interval. Please clarify whether it refers to the AUC at a dosing interval at steady state or after a single dose as the two are different.	It is considered that the guideline is clear about the requirement of a clamp study investigating the PK and PD profiles of single doses of insulin.
256-259	1	Comment: To our knowledge, there are no examples of products where a wider range than the 80-125% would be acceptable for insulin products. For insulins where specific assays can be developed, a wider range would be unacceptable. Unless some good and relevant examples can be referenced, it is suggested to delete line 256-260.	Not accepted. Literature suggests that, for example, isophane insulins have high intra- and inter-subject variability. In such cases and in analogy to the bioequivalence guideline for generics, a replicate design study may be used to justify wider acceptance ranges.

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		Proposed change (if any): If high variability is anticipated, a replicate design study should be considered (e.g. 3-period cross-over design with replication of reference) to justify widening of the acceptance range.	
256-258	3	**Comment:** There is growing evidence on PK/PD variability of insulins and underlying mechanisms. For example, in a recent study with insulin aspart (Rasmussen et al., Eur J Pharm Sci 2014; 62C: 65-75), using techniques such as visualisation of subcutaneous insulin injections by X-ray computed tomography, it was demonstrated how the subcutaneous route of administration impacts PK variability. It was shown that antibody binding, oligomeric transitions in subcutis, and blood flow dependent variations in absorption rate all contribute to enhanced PK variability. Therefore we suggest to clearly specify subcutaneous route as a typical example of situation where a widened acceptance range for bioequivalence could be considered. **Proposed change:** If high variability is anticipated (e.g. in subcutaneous administration route), a replicate design study should be considered (e.g. 3-period cross-over design with replication of reference) to justify widening of the acceptance range (…).	Not accepted. Most insulins do not exhibit high intra-individual variability with s.c. use. On the other hand, isophane insulins have been reported to have high variability. The guideline is clear in that, if a widening of the acceptance range is intended, this should be supported by data from a replicate design study.
259-260	11	Comment: The current wording requires bioequivalence margins for total and maximum exposure (AUC and Cmax), but not for secondary endpoints. In order to show comparable PK profile and	Not accepted. There is no requirement for secondary endpoints to meet the acceptance criteria predefined for the primary endpoints. Nevertheless, it is clear that all data will be considered.

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		biologic effect secondary PK and PD endpoints should also be comparable between the biosimilar and reference product. If not, e.g. a rapid-acting insulin product with a left or right shift (faster/slower initial absorption) in the PK time profile could wrongly be considered bioequivalent if total and maximum concentration 90% CI within 80-125% are the only criteria. Proposed change (if any): For the other parameters descriptive statistics would be appropriate or statistical analysis could be used, as appropriate. The evaluation of biosimilarity in PK/PD will be made based on the totality of the PK/PD profiles.	
260	1	Comments: In line 274-275 it is stated “In case a replicate design study is performed, intra-individual variability should also be documented for PD endpoints.” Presumably this should be relevant also for PK parameters and it is suggested to add a similar statement to the PK section. Proposed change (if any): Add to line 260: In case a replicate design study is performed, intra-individual variability should also be documented.	Not accepted. It is evident form the text that the purpose of the replicate design study is to estimate the intra-individual PK variability and, in case it is confirmed to be high, to support a widening of the acceptance range.
262-267	1	Comment: GIR could be standardized for body weight. Body weight is mentioned in the context of insulin doses, where it is assumed that dosing is per kg body weight. For consistency, GIR should be standardized	Not accepted. Not considered necessary in a cross-over study.

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		for body weight as well, though this may be less important in a crossover setting and with log transformation. Proposed change (if any): Add to line 267: Standardization of GIR for body weight should be considered.	
273-274	1	Comment: It is stated that for primary GIR parameters, equivalence margins should be pre-defined and justified. It is suggested to clarify whether this applies if, as described in the previous paragraph (lines 268-271), all GIR-related parameters are defined as secondary endpoints. Proposed change (if any): For primary GIR parameters used as primary endpoints, equivalence margins should be pre-defined and justified.	Accepted. The issue has been clarified.
274	1	Comments: There is currently no guidance for the choice of equivalence margins for PD endpoints. If added, it is suggested to state that the established bioequivalence margins of 80-125% should not be exceeded.	Not accepted. If PD parameters are primary endpoints, equivalence margins have to be justified.
276-289	3	**Comment:** Guidance given within the 'Quality of insulin clamps' subtitle is acknowledged. However, because this guideline document is product-specific, in order to optimally apply this guidance, a closer approach to definition of "noise" of GIR (but also PK)	Not accepted. The guideline already provides rather detailed guidance.

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		measurements is needed. The existing body of evidence could allow a closer definition of outliers (outlying values) for insulin clamps.	
291-298	1	Comment: The section on long acting insulins describe many of the problems connected to comparing long-acting insulins in the same way as short or intermediate acting. However, it may be instructive to also include some more text on the viable alternatives. For obtaining correct pharmacokinetic and pharmacodynamic data for a long-acting insulin analogue, steady state data are required. This can only be obtained in a multiple-dose study using the intended dosing frequency. The following additions and changes are therefore proposed for the first paragraph in the section “Specifics of long-acting insulin preparations”: Proposed change (if any): Long-acting insulin preparations are intended to produce a time-concentration profile that, as far as possible, approximates physiological basal insulin secretion. For a long-acting insulin preparation, a single-dose study may not be adequate to determine the clinically relevant properties, such as shape of profiles (distribution across one dosing interval), AUCs, steady state exposure level and glucose-lowering effect, and duration of action. In such cases, a multiple-dosing study should be performed using the clinically relevant dosing frequency. The time-concentration insulin profile and the time-glucose infusion rate profile over	Not accepted. See comment above.

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		one dosing interval at steady state should be presented and endpoints described in the endpoint section evaluated. The dose used should be clinically relevant. Due to their flat pharmacokinetic profile, determination of Cmax and tmax (for insulin and GIR) may not be possible and may be meaningless. The duration of the clamp should be sufficient to be able to evaluate the duration of action and, as a minimum, be as long as the intended length between doses (one dosing interval) in the clinical setting. Due to the slow decline in insulin action, together with the unavoidable variations of the GIR, especially in the “tail part” of the GIR curve, it may be difficult to determine the duration of action of a long-acting insulins, particularly in healthy subjects with interfering endogenous insulin. Therefore, patients with type 1 diabetes are generally considered more suitable to determine the time-action profile of long-acting insulins.	
319-320	1	Comment: It is suggested this section should acknowledge that lack of similarity based on non-clinical and PK/PD work cannot be overcome by showing a lack of difference in clinical efficacy outcomes. This point seems to be implied in current wording, but it is suggested to make it explicit. Proposed change (if any): There is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not considered	Partly accepted. Second sentence considered superfluous.

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		sensitive enough for the purpose of showing biosimilarity of two insulins. Consequently, lack of similarity based on non-clinical and PK/PD work cannot be overcome by a lack of difference in clinical efficacy outcomes.	
319-320 Clinical efficacy	2	**Comment:** EBG fully agrees with the statement in this section that apart from the comparative pharmacokinetic and pharmacodynamic assessment there is no anticipated need for separate efficacy studies. The justification for this is the lack of efficacy endpoints sensitive enough to detect clinically relevant differences between two insulins. **Proposed change: '**There is no anticipated need for specific efficacy studies since endpoints used in such studies, usually HbA1c, are not sensitive enough for the purpose of **detecting clinically relevant differences between** ~~showing biosimilarity of~~ two insulins.'	Accepted
322	1	Comment: To avoid that the sentence "Generally, safety studies should be performed with specific focus on immunogenicity." is interpreted as a possibility for approval of a biosimilar without any clinical study it is suggested to delete the word "Generally". Proposed change (if any): Generally, safety studies should be performed with specific focus on immunogenicity.	Not accepted. In fact, the guideline opens up the possibility that, in certain cases, a pre-licensing safety study including immunogenicity assessment may be waived and states the conditions for waiving such a study.

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326-328	1	Comment: The safety concerns with a biosimilar insulin relate mainly to the potential for immunogenicity. The issue of immunogenicity can only be settled through clinical trials of sufficient duration. As stated in the guideline, anti-drug antibodies, if any, often develop early on and a pre-approval study of 6-months is therefore acceptable. However, as anti-drug antibody peaks have been reported between 3 and 12 months after initiation of insulin (see Lindholm, Diabetes Care, 2002; and Fineberg, Diabetes Care, 2003), 12 month data should also be generated. Proposed change (if any): "The issue of immunogenicity can only be settled through clinical trials of sufficient duration, which for subcutaneous administration is at least 12 months. Since anti-drug antibodies, if any, usually develop early on, a 6-month study investigating incidence and titres of antibodies to the test and reference medicinal products should be performed completed pre-approval. Data at the end of 12 months could be presented as part of post-marketing commitment."	Not accepted. The majority of anti-drug-antibodies is expected to develop within the first 6 months of treatment.
328-331	1	Comment: As there is no generally accepted sample size calculation to power the study to formally demonstrate non-inferiority regarding immunogenicity, we suggest that all studies should be sufficiently powered to demonstrate non-inferiority based on HbA1c as primary endpoint.	Not accepted. Other sample size calculations may be acceptable, if adequately justified.

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		Proposed change (if any): However, there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity and, if considered desirable by the sponsor, it would be acceptable to calculate Formal demonstration of non-inferiority is not necessary. However, sample size should at a minimum be calculated based on an efficacy-oriented endpoint (such as HbA1c).	
328-331	4	It is agreed that there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity. However, the confidence intervals should be rather narrow for enabling a proper judgment of non-inferiority. In addition, calculating the sample size upon an efficacy endpoint is not advisable if it prevents a properly powered study on immunogenicity. Proposed change (if any): However, there is no need to power the study to formally demonstrate non-inferiority regarding immunogenicity and, if considered desirable by the sponsor, it would be acceptable to calculate sample size based on an efficacy-oriented endpoint (such as HbA1c). However, the confidence intervals should be sufficiently narrow for enabling a proper judgment of non-inferiority.	Partly accepted. A sentence has been included that the size of such a study is expected to reasonably exclude clinically relevantly increased immunogenicity.
334-335	4	The sentence states “If a background insulin is given	Potential for misunderstanding acknowledged.

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		during the trial (e.g. an approved prandial or basal insulin in addition to the test insulin), this should not be changed during the evaluation period." The insulin should indeed not be changed, however the dose cannot be fixed as it depends on the study-protocol whether the dose should be kept between margins or whether this should be titrated to target.	The sentence has been modified to read "…..the type and regimen of the background insulin should not be changed during the evaluation period."
335-339	1	Comment: In case of development of different preparations of a biosimilar containing the same active ingredient, a single safety study using only the solution may be adequate, e.g. if the other ingredients of the biphasic preparation (i.e. protamine) have been studied previously and are therefore well-known, and the solution will be investigated in a pivotal study. It is assumed that PK / PD studies in the biphasic preparation will be performed by the manufacturer. Proposed change (if any): In case a biosimilar manufacturer develops different preparations, e.g. short-acting, intermediate-acting and biphasic preparations containing the same active ingredient, only a single safety study is usually required using either all of these preparations can be adequate, alternatively, only the biphasic preparation or only the free combination of short and intermediate-acting each formulation should be investigated in a safety study vs. preparations and its respective reference product. PK / PD studies are considered mandatory for the biphasic preparation.	Not accepted. Only one immunogenicity study is required for different preparations containing the same active ingredient. The guideline has been modified to clarify that the preparation with the highest expected immunogenic potential should be included in the safety/immunogenicity study.

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342-350	1	Comment: Although prerequisites described in the draft guidance for the pre-licensing study waiver would provide reassurance, we consider that evaluation of immunogenicity as well as similarity of the adverse event profile regarding hypoglycaemia and local tolerability should be confirmed with a pre-licensing safety study. Proposed change (if any):	Not accepted. The approach delineated in the guideline is considered scientifically sound.
353-354	1	Comment: Identified risks for the reference product should not be the only safety concerns followed for the biosimilar product. If other safety concerns are observed during the biosimilar product development program, these should also be considered. The biosimilar is not identical to the originator product; therefore, there may be safety issues unique to the biosimilar. The main safety concerns for insulin preparations are already known: hypoglycaemia, local reactions, hypersensitivity reactions and antigenicity. To give clearer guidance, the above events should be indicated as a minimum set for safety specifications in the RMP. Proposed change (if any): “The RMP of the biosimilar should always take into account identified and potential risks associated with the use of the reference product, such as hypoglycaemia, hypersensitivity reactions etc. In addition, safety concerns observed	Not accepted. It is clear that a safety study will evaluate all adverse events. The guideline states that the risk management plan (RMP) of the biosimilar should always take into account identified and potential risks of the reference product. This does not exclude that other minor uncertainties (e.g. more accurate estimate of antibody development) are addressed in the RMP. In case of additional identified risks of the test product, biosimilarity would be highly questionable.

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		during the biosimilar product development program should also be considered. ”	
357-362 Extrapolation of indications	2	**Comment:** EBG agrees to and highly appreciates this new section confirming the basic concept for extrapolation of indications. Namely, the totality of evidence provided for the biosimilar development should be considered as the scientific basis for extrapolation of indications. More specifically, justification for extrapolation depends on the comprehensive structural and functional similarity (including in vitro non-clinical data) of the biosimilar to the reference product, the pharmacokinetic and, where needed, pharmacodynamic similarity of parameters that are sensitive enough detecting potential clinically relevant differences taking into account the mechanism (s), the clinical experience and the available literature data. The structure and functions of insulin are susceptible to full characterisation. As it is not a glycoprotein, its active substance is also a single moiety which will be identical for both the biosimilar and its reference product. Extrapolation to all uses and indications of the reference product is therefore straightforward provided the data regarding structure, biological functions, and PK/PD profiles demonstrate a lack of any clinically relevant differences. **Proposed change:** ‘**Provided that biosimilarity between the biosimilar and the reference insulin can be convincingly concluded from the**	Partially accepted. The concept of the “totality of evidence” has been captured in the modified version.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		physicochemical and functional characterisation and comparison using sensitive, orthogonal and state-of-the-art analytical methods, ~~D~~**d**emonstration of similar pharmacokinetic and, where needed, pharmacodynamic profiles of the biosimilar and the reference product and absence of safety issues with subcutaneous use will allow extrapolation of these data to intravenous use, if applicable, and to other indications and patient populations licensed for the reference product.'	
358-361	1	Comment: To say that similar PK/PD profiles "will" allow for extrapolation to other indications and patient populations of the reference product seems to broad and absolute. Extrapolation should be based on the totality of evidence provided from the comparability exercise. Applicants should provide a well-developed, science-based justification to support extrapolation. Proposed change (if any): Demonstration of similar pharmacokinetic and, where needed, pharmacodynamics profiles of the biosimilar and the reference product and absence of safety issues with subcutaneous use will may allow extrapolation of these data to intravenous use, if applicable, and to other indications and patient populations licensed for the reference product, provided extrapolation is otherwise scientifically justified.	Partly accepted. The general guidelines already highlight that the conclusion of biosimilarity will always be based on the totality of data/evidence. This concept has now also been made clearer in the insulin guideline. If biosimilarity has been demonstrated for an insulin, extrapolation to other populations and from SC to IV use is considered possible. In addition, there are no anticipated safety concerns that would not be detectable by analytical methods or with SC use.
361-362	4	Comment: It is stated that "If a rapid- or a short-acting biosimilar	Not accepted. Beyond the scope of the guideline.

Line no.	Stakeholder no.	Comment and rationale; proposed changes	Outcome
		insulin is intended for use in pumps, additional stability data may be required." There are multiple issues to be addressed when the biosimilar is intended for use in pumps, stability being one example. It is recommended to amend the sentence as follows: Proposed change (if any): "If a rapid- or a short-acting biosimilar insulin is intended for use in pumps, there are additional quality related issues to be addressed, e.g. additional stability data may be required."	