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November 22, 1999

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Office of Generic Drugs
Center for Drug Evaluation & Research
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Docket No. 99D-2729

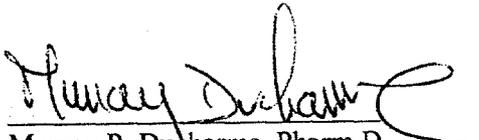
Re: FDA draft guidances on bioequivalence

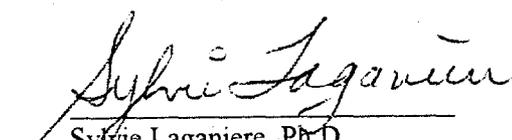
Dear Dr. Connor,

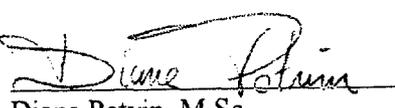
Please find enclosed our comments and suggestions regarding the two draft guidances for industry entitled "BA and BE studies for orally administered drug products - general considerations" and "Average, Population and Individual approach to establishing bioequivalence". We have already sent these comments to the NAPM and the GPIA science committee to help them draft their own responses to the FDA. Since they only used part of these comments, we felt it was appropriate to send you our complete document.

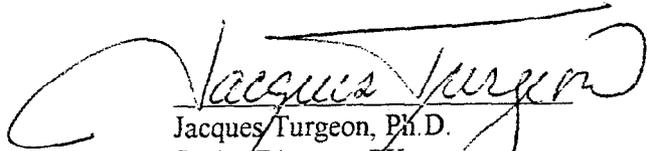
We hope that these comments will be helpful to you and the FDA in the development of the final guidances. Please do not hesitate to contact us if you need any additional information.

Sincerely,


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cc: Dr. Mei-Ling Chen, Food and Drug Administration

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OVERALL ASSESSMENT OF THE GUIDANCE entitled "BA and BE studies for orally administered drug products-General considerations".

This guidance incorporates a large number of new and innovative ideas for the conduct of BA/BE studies. Since these changes will impact on the results of BA/BE studies, it is worthwhile evaluating carefully the scientific rationale underlying these new concepts. Several comments are included in the following document in order to put these new ideas into perspective and hopefully improve the proposed guidance.

For clarity purposes, we will address some of these new concepts included in the guidance point by point.

Comment No. 1

III. METHODS TO DOCUMENT BA AND BE

A. Pharmacokinetic studies

4. Replicate study design

The actual guidance proposes that:

Replicate study design (see section IV) are recommended for all BE studies using pharmacokinetic measurements with the following exceptions: ...

Proposed change:

*Replicate study design (see section IV) are recommended for **BE studies involving drugs associated with large within-subject variability**, using pharmacokinetic measurements with the following exceptions: ...*

Rationale:

Replicate study designs are proposed for drugs with large within-subject variability. Indeed, statistical models can be developed for studies with a replicate design to differentiate within-subject variability independently of inter-subject variability, intra-subject variability due to switchability, and residual variability. In non-replicated studies, all these sources of variability are confounded to some extent. For drugs associated with a small within-subject variability, the impact of confounded variances is minimal on the conclusion of BE studies and does not support the mandatory use of a replicate design.

Comment No. 2

III. METHODS TO DOCUMENT BA AND BE

B. Pharmacokinetic studies

5. Study population

The actual guidance proposes that:

An attempt should be made to admit as heterogeneous a study population as possible, with a reasonable balance of males and females, young and elderly, and members of different racial groups.

Proposed change:

Remove entirely this sentence.

Rationale:

It is very important to characterize the clinical pharmacology of new drugs in as many different sub-populations as possible. However, the purpose of such studies in the BE context, would merely consist of the detection of the subject by formulation interactions in some of those sub-populations. Although this is worth assessing, studies should not be designed to include all these sub-populations at the same time.

1. Mixing sub-populations will increase inter-subject variability and may prevent accurate characterization of the pharmacokinetics of the drug formulations in these separate sub-populations. It seems more reasonable to characterize the pharmacokinetics of drugs separately in each sub-population in order to get robust results.
2. Mixing sub-populations will also increase the risk of detecting multi-modal distributions among the pharmacokinetic parameters. Consequently, statistical analysis will have to deal with major issues concerning transformation of multi-modal distribution of PK parameters
3. If replicate designs are to be used, it is likely that multiple groups will be required in order to complete the panel for a BE study. The increased inter-subject variability within the groups will also increase the chances of detecting a group effect preventing merging of the data necessary for the complete pharmacokinetic and statistical analysis of the data.
4. Selection of alternates will also be complicated by the number of combinations possible due to the inclusion of subjects from three to four sub-populations. These subjects will also have to be matched for sequences according to potential dropouts. This is again creating a significant burden if replicate designs have to be used due to the number of sequences possible.

5. The assumption of time-independence behavior in the elimination of a drug may not be valid in some subgroups, and will have to be dealt in a special manner. Elderly people may present a renal or a hepatic function that changes with time. Women may be given a drug that is eliminated through an enzymatic pathway dependent on the menstrual cycle.
6. Volume of blood withdrawn during studies with a replicate design may become of concern in some sub-populations such as elderly and women.
7. The inclusion of elderly people free of any medication will be challenging. If elderly persons on current medications are to be included, complete management of drug compliance will be required for the entire duration of the study (which can be as long as two months for replicate designs).
8. On a logistic point of view, it may become impossible to conduct BE studies in man, woman, and elderly people at the same time. If studies have to be conducted on different days, more complex statistical analyses will be needed.

Please see additional comments on page 14 regarding statistical issues related to this topic.

Comment No. 3

III. METHODS TO DOCUMENT BA AND BE

A. Pharmacokinetic studies

8. Pharmacokinetic Measures of Systemic Exposure

The actual guidance proposes that:

This guidance therefore recommends a change in focus from these direct or indirect absorption rate measurements to measurements of systemic exposure. The change in emphasis allows continued use of C_{max} and AUC as product quality BA and BE measurements ... as follows.

a. Early exposure

Early exposure in a product quality BA study can be assessed by measuring the partial area under the concentration time profile curve with a cutoff at the peak time (T_{max}) of the drug. To establish BE, the partial AUC is truncated at the time of the peak of the reference formulation in each subject.

Comments on this new concept:

The guidance proposes that we compare reference and test formulations using up to three parameters (AUC_{0-T_{max}}, C_{max}, and AUC_{0-inf}). The purpose of a BE study is to compare two formulations in terms of their rate and extent of absorption. In

pharmacokinetics, these processes are described by the parameters K_a (absorption rate constant) and F (bioavailability). Since individual pharmacokinetic analysis using compartmental methods is very susceptible to noise in a data set, pharmacokineticists have used the noncompartmentally derived parameters $AUC_{0-\infty}$ (extent of absorption) and the C_{max} (rate of absorption). However, C_{max} is limited in its ability to describe the rate of absorption of a drug. In addition, its value is determined by a single concentration-time point making it susceptible to experimental error. Calculating a partial area under the concentration-time profile curve with a cut-off at the T_{max} of the reference product is proposed in the new guidelines. Unfortunately, this calculation will not give accurate results in the following instances:

1. The drug is associated with a lag-time before the absorption process starts. This is very common with orally administered drugs, so much that it is usually not appropriate to determine the pharmacokinetics of a drug with compartmental methods not taking into account this parameter. Drugs associated with a significant lag-time usually demonstrate a very large within-subject variability in this parameter (ex. cyclosporine, omeprazole) but not necessarily in the absorption parameters themselves (K_a). With drugs associated with a lag-time, giving the same drug formulation to the same individual will result in a different T_{max} .
2. The guidance proposes the use of fully replicated designs. In such studies, T_{max} may not be similar between periods for the same formulation.
3. Drugs with multiple absorption peaks will obviously cause problems in determining the T_{max} to be used for the partial AUC calculation.
4. Modified release drugs can present very variable T_{max} even though plasma concentrations are not variable. Consequently, large intra and inter-subject variability will be observed in the T_{max} value used for the calculation of the AUC.
5. The sampling schedule will clearly dictate exactitude of the data obtained. Indeed T_{max} is not an independent variable, but dictated by the protocol and therefore is the partial AUC.

In view of these comments, we cannot support the proposed partial AUC approach. Alternatives based on a similar concept should be tested prior to implementation.

Comment No. 4

IV. COMPARISON OF BA MEASURES IN BE STUDIES

The actual guidance proposes that:

This guidance recommends that certain in vivo BE studies conducted for (1) INDs, (2) NDAs, (3) ANDAs, and (4) amendments and supplements to NDAs and ANDAs be conducted using replicate designs (see section III.A.4). Sponsors

may analyze their data using average or population BE criteria (INDs and NDAs) or average or individual BE criteria (ANDAs and supplements to NDAs and ANDAs), provided the choice is specified in the study protocol prior to study initiation. At the sponsor's discretion, scaling may be used to judge BE when either an individual or population BE criterion is specified. When a replicate fasting study is infeasible, sponsors are encouraged to contact appropriate review staff. In specified circumstances, replicate study designs are not needed.

Comments on this new concept:

Pharmacokinetic studies used to compare the BE of two formulations A and B involve the following types of variability:

1. The inter-individual variability present with formulation A.
2. The inter-individual variability present with formulation B.
3. The intra-individual variability present with formulation A.
4. The intra-individual variability present with formulation B.
5. The residual experimental error or noise.

With ABE, the variability 3), 4) and 5) are confounded in a single variability measurement. In addition, contamination of 1) and/or 2) with the residual variability is possible. This approach for BE studies increases the burden on generic drugs when the reference or both drug formulations are highly variable.

With IBE, the intra-individual variability for formulations A and B can be estimated preventing the contamination of the inter-individual variability. However, the variability 3) and 4) are still confounded with 5). Therefore, there is a potential for the variability 3) to be different from 4) not because of a difference between formulations, but because of the contamination. This is likely to be observed with C_{max} , since this parameter is determined using only one concentration data-point. Indeed, it remains impossible in a given subject to differentiate between residual variability (i.e. analytical assay error) and real within-subject variability.

IBE also allows for an assessment of subject by formulation interaction and potential switchability issues. Although data indicate that these effects are rarely observed, it may still be of interest to include them in the model in order to decrease consumer risk.

Scaling has been proposed in the guidance as an approach to control for variability associated with the reference product. Retrospective analysis of studies performed using a replicate design clearly indicated that IBE with reference scaling is the favorable approach when the CV of the reference product is greater than 25%. IBE is also a favorable approach if variability

of the reference product is equal to or greater than variability associated with the test product. Finally, IBE with reference scaling decreases consumer risk when the test product is more variable than the reference product. Thus, observations from data on file suggests that IBE with reference scaling offers advantages when variability of the reference product is greater than 25% and when no switchability issue are observed.

Comment No. 5

V. DOCUMENTATION OF BA AND BE

C. Immediate-Release Products: Capsules and Tablets

1. *General recommendations*

The actual guidance proposes that:

For BE studies for immediate-release dosage forms where the drug product contains a narrow therapeutic range drug (see section VI.F), this guidance recommends the following: 1) where an average BE criterion is selected, use of a BE limit of 90-111 percent for AUC; 2) where an individual BE criterion is selected, reference scaling is recommended, regardless of the variability of the reference listed drug. In addition, this guidance recommends that the allowable upper limit be calculated with $\epsilon_j=0$ (i.e., $\theta=1.245$).

Comments:

Drugs listed in section VI.F as narrow therapeutic range drugs were introduced to the market several years ago. The notion of risk with these compounds may rather reflect a poor understanding of the pharmacokinetics of these drugs at the time they were first used. Other compounds have been introduced to the market recently exhibiting complicated pharmacokinetics (racemic compounds, non-linear pharmacokinetics, drugs metabolized via polymorphically distributed enzymes, drugs with active metabolites, etc...). A clear definition of narrow therapeutic range drugs should be provided before implementation of this guidance.

Pharmacokinetics of some of the drugs listed as narrow therapeutic range drugs are described by non-linear pharmacokinetics (phenytoin) or are racemic compounds containing enantiomers that differ in their activity (warfarin). Determining the BE of formulation of a drug displaying non-linear pharmacokinetics is a different but challenging issue that should be given consideration at the FDA. We will discuss the case of racemic compounds later on in this document.

The limit on the acceptable extrapolation of the AUC parameter with the elimination half-life should be specified. It is routinely accepted that AUC should not be extrapolated at more than 20% of their value with the elimination half-life (i.e. AUC_{0-T} is 80% or more of AUC_{0-inf}). The uncertainty associated with the calculation of the AUC is roughly equivalent with the percentage of extrapolation (i.e. 20% extrapolation is associated with a 20% uncertainty in the calculation of the AUC). Therefore, if one wants to determine the bioequivalence of two formulations at a BE limit of 90-111, the AUC should not be more than 10% extrapolated.

It can be assumed that narrow therapeutic drugs on the market exhibit small within-subject variability otherwise plasma concentrations outside of the therapeutic range would be observed regularly. Use of reference scaling in the model for drugs described as narrow therapeutic drugs (even for drugs with CV% less than 25%) should control for potential consumer risk in BE studies with these compounds. Therefore, arbitrary change of the confidence interval limits or θ_1 in addition to reference scaling should not be required.

Comment No. 6

V. DOCUMENTATION OF BA AND BE

C. Immediate-Release Products: Capsules and Tablets

1. *Exposure measurements*

The actual guidance proposes that:

At the request of a sponsor or the reviewing division, application of partial AUC as an early exposure measurement may be justified on the basis of appropriate clinical safety and/or efficacy trials and/or PK/PD studies (see section III.A.8).

Comments:

We have already mentioned the limitations associated with the use of partial AUC as an early exposure measurement in the previous pages of this document. Use of the partial AUC method does not offer a robust assessment of the rate of absorption of drugs in certain instances.

Comment No. 7

V. DOCUMENTATION OF BA AND BE

D. Modified-Release Products

The actual guidance mentions that:

Delayed-release drug products are dosage forms that release the drugs at a time later than immediately after administration (i.e., these drug products exhibit a lag time in quantifiable plasma concentration) ... In vivo requirements for delayed-release drug products are similar to extended-release drug products.

Comments:

Most drugs administered via PO administration exhibit a lag-time before the beginning of drug absorption. This lag-time may be very short and therefore of little overall influence on the T_{max}. Lag-time are not only associated with delayed-release drug products. This has great influence on the method that needs to be used to characterize adequately the pharmacokinetics of a compound. In vivo assessment of BE between two drug formulations that exhibit a lag-time cannot be performed reliably using the T_{max} or the partial AUC (see comment No. 3 for the complete explanation).

Comment No. 8

V. DOCUMENTATION OF BA AND BE

D. Modified-Release Products

2. *ANDAs: BE Studies*

The actual guidance mentions that:

For drugs that exhibit nonlinear kinetics and/or drugs designated as narrow therapeutic range drugs (see section VI.F), this guidance recommends the following: (1) where an average BE criterion is selected, use of a BE limit of 90-111 percent for AUC; (2) where an individual BE criterion is selected, reference scaling is recommended, regardless of the variability of the reference product. In addition, this guidance recommends that the allowable upper limit be calculated with $\epsilon_1=0$ (i.e., $\theta_1=1.245$). Where a replicate fasting study is infeasible, sponsors are encouraged to contact appropriate review staff.

Comments:

Two formulations of a drug exhibiting non-linear pharmacokinetics cannot be reliably compared in terms of BE with noncompartmental pharmacokinetic approaches (i.e. AUC, C_{max}, partial AUC). Linear pharmacokinetics is a fundamental assumption of noncompartmental pharmacokinetics. The arbitrary setting of a BE limit of 90-111% for AUC for drugs exhibiting non-linear pharmacokinetics is irrelevant and not scientifically sound.

Comment No. 9

V. DOCUMENTATION OF BA AND BE

D. Modified-Release Products

3. Exposure Measurements

The actual guidance mentions that:

This guidance recommends that early and total exposure measurements be analyzed in single-dose studies for modified-release drug products.

Comments:

In addition to all the previously mentioned reasons limiting the usefulness of the partial AUC method, the early exposure measurements will not be a robust estimate of the rate of absorption of a modified-release drug formulation for the following reasons:

1. These drugs very often exhibit lag-times before the beginning of drug absorption. We have already mentioned the limitations of the partial AUC method in this circumstance (see previous comments).
2. Concentration-time profiles of modified-release products are frequently associated with concentrations changing little over time during the absorption process. Timing of the peak concentration may be highly variable with these formulations due to experimental errors associated with any plasma concentration. If the T_{max} is not adequately characterized, the partial AUC method will not be appropriate.

Comment No. 10

VI. SPECIAL TOPICS

B. Moieties To Be Measured

1. Parent Drug Versus Metabolites

The actual guidance mentions that:

The moieties to be measured in BA and BE studies are the active drug ingredients or active moiety in the administered dosage form and, when appropriate, its active metabolites (21 CFR 320.24(b)(1)(I)). This guidance recommends the following approaches for BA and BE studies:

For BE studies, determination of only the active moiety and/or active ingredient in the dosage form, rather than the metabolite, is generally recommended. The rationale for this recommendation is that the concentration-time profile of the active moiety in the dosage form is more sensitive to changes in formulation performance than a metabolite, which is more reflective of metabolite formation, distribution, and elimination. The following are exceptions to this general approach.

Comments:

The proposed modification in this guidance is scientifically sound. However, a ratio of the AUC of a major metabolite over that of the parent compound should be calculated for specific drugs. An arbitrary limit should be set based on NDA data in order to determine whether the metabolite or the parent compound should be determined in order to establish BE. As well, emphasis should be put on the metabolite under conditions where a pro-drug is administered orally.

Comment No. 11

VII. SPECIAL TOPICS

B. Moieties To Be Measured

2. *Enantiomers Versus Racemates*

The actual guidance mentions that:

For BA studies, measurements of both enantiomers may be important. For BE studies, this guidance recommends measurements of the racemate using an achiral assay, without measurement of individual enantiomers. However, measurements of individual enantiomers in BE studies is recommended when all of the following conditions are met: (1) the enantiomers exhibit different pharmacodynamic characteristics; (2) the enantiomers exhibit different pharmacokinetics; (3) the primary activity resides with the minor enantiomer, defined as having <20 percent of the total of all the enantiomer AUC; and (4) nonlinear absorption is present (as expressed by a change in the enantiomer's concentration ratio with change in the input rate of the drug) for at least one of the enantiomers. In such a case, BE criteria should be applied to both enantiomers.

Proposed changes:

Condition (4) should be removed since it will be extremely difficult to prove experimentally whether or not enantioselective non-linear absorption is observed using noncompartmental analysis. The concept behind condition (3) is sound but the 20% limit appears very relaxed (40% would seem more reasonable). As well the guidance should indicate whether this value is derived from oral or intravenous administration of the drug. BE of compounds meeting conditions (1), (2) and a modified (3) should be compared in

terms of the individual enantiomers and not the racemate. Differences in the absorption process and/or the first-pass metabolism of different enantiomers may be associated with differences in the AUC and Cmax of the total drug concentrations depending on the individual formulation performance of the drug.

Comment No. 12

VII. SPECIAL TOPICS

C. Long Half-Life Drugs

The actual guidance mentions that:

For BE determination of long half-life drug products, a nonreplicate, single-dose, crossover study can be conducted, provided an adequate washout period is used. If the nonreplicate, crossover study is problematic, a parallel BE study design can be used. For a crossover or parallel study design, sample collection time should be adequate to ensure completion of the drug product's gastrointestinal transit (approximately 2 to 3 days) and drug absorption. In addition, if the drug distribution and elimination are similar for the two products (i.e., intra/intersubject variation is low), Cmax and a suitably truncated AUC should be used to adequately characterize the rate and extent of absorption. Alternatively, whenever intra/intersubject variations in distribution and elimination are high, truncated AUCs should result from a similar amount of truncations for each subject's plasma concentration-time curve.

Comments:

This paragraph appears to suggest that drug distribution and elimination can be different for two products if intersubject variation is high. This concept needs to be demonstrated since pharmacokinetic models predict that the same active ingredient from two different formulations following absorption will always demonstrate exactly the same distribution (i.e. Vc, Vp, etc... which is different than Vc/F or Vp/F) and elimination (i.e. CL which is different than CL/F). Only intra-subject variability will result in a different elimination and distribution characteristics in a given subject.

Using a similar amount of truncation for a truncated AUC in replacement of the AUC_{0-inf} for long half-life drugs is an interesting concept which has been debated in the literature for some time. The Canadian TPP are suggesting the use of an AUC₀₋₉₆ for drugs associated with an elimination half-life of 12 hours or more. The current FDA guidance needs to be clearer in terms of their cutoff for the elimination half-life, and in terms of the minimum amount of time necessary for the truncated AUC to be robustly indicative of the AUC_{0-inf}. A truncated AUC₀₋₄₈ or AUC₀₋₇₂ appears to be suggested.

Comment No. 13

APPENDIX 2: General Pharmacokinetic Study Design

Subjects with predose plasma concentrations:

The actual guidance mentions that:

If the predose concentration is less than or equal to 5 percent of C_{max} value in that subject, the subject's data can be included in all pharmacokinetic measurements and calculations. If the predose value is greater than 5 percent of C_{max} , the subject should be dropped from all BE study evaluations.

Comments:

Predose concentrations can be due to several factors. Possible causes are:

- 1) An analytical interference.
- 2) A drug that is an endogenous substance is administered. Two different situations may arise. A) The endogenous release of the drug is not affected. B) The endogenous release of the drug is affected by negative feedback.
- 3) The subject was already taking the drug before the beginning of the study.
- 4) Obviously the guidance should specify that these recommendations do not apply to multiple dose studies.

All of these situations have to be dealt in a different and specified manner.

OVERALL ASSESSMENT OF THE GUIDANCE entitled "Average, Population, and Individual approaches to establishing bioequivalence".

V. Study design

A. Experimental Design

- The proposed replicated crossover design with four periods and two sequences TRTR, RTRT is an efficient design when a "standard" bioequivalence study is performed where no carryover of the drugs can be expected from the previous period. However, the following design RTTR, TRRT is more efficient than the one proposed in the draft guidance in the presence of carryover and as efficient as the one proposed under no carryover assumptions. Therefore, the use of RTTR and TRRT¹ as sequences for replicated designs should be favored.
- A three-period, 2-sequence design (semi-replicated design) to show bioequivalence using the proposed IBE metric is not appropriate². Individual criterion is based on the comparison of an expected squared distance between T and R formulations (administered to the same subject) to the expected distance between two administrations of R formulation (two administrations of R to the same subject). In a semi-replicated design, these expected squared distances are not defined for every subject in every sequence. Moreover, the method described in Appendix H to derive the upper limit for the IBE metric is only applicable for a four-period design with equal replication of T and R in each of s sequences. This method cannot be adapted to a 3-period, 2 sequence semi-replicated design.

B. Study Population

Please see additional comments on page 3 regarding this topic.

- The use of an heterogeneous sample will modify the statistical model used to perform the ANOVA. Main factors such as gender, race, age, etc. might be introduced into the model along with some interesting interactions such as formulation*race, formulation*gender, etc.

If none of these interactions are statistically or clinically relevant, then the analysis will be quite straightforward and the principal advantage of including the main factors will be to get better (smaller) estimates of the between-subject variances for the T and R formulations.

However, if some interactions are clinically relevant, bioequivalence will be difficult to demonstrate. If bioequivalence (using ABE, IBE or PBE) has to be demonstrated inside each relevant subgroup, the power might be dramatically

decreased. Sample size evaluation will have to be adjusted for this possibility. Can bioequivalence be demonstrated in pooling the subgroups (in ignoring the significant interactions)? The variance due to a lack of switchability (σ^2_D) will be increased and will affect all three criteria (ABE, IBE and PBE), but at different degrees.

It is quite possible to have instances where the analysis by subgroup shows bioequivalence using IBE in every subgroup (given sufficient power) and the analysis where subgroups are pooled shows non-bioequivalence using IBE. Knowing that some interactions are observed for some subgroups, should bioequivalence be shown within every subgroup or the whole sample pooled together?

C. Sample size and dropouts

- The draft guidance stipulates that “The number of subjects for BE studies based on either PBE or IBE should be estimated by simulation, because analytical approaches for estimation are not available” and gives some samples size calculations based on simulated data in Appendix C.

A formula for sample size calculation based on a F distribution and the weighted version of the FDA metric proposed by Kimanani and Potvin³ can easily be derived. Based on this formula for both the unweighted (FDA) and the weighted IBE metric, consistent results were obtained. These results are somewhat in contradictions with what is presented in Appendix C for the estimated recommended numbers of subjects for IBE. Sample size estimations based on a F distribution decreases with increasing within-subject variance (see Tables 1 and 2 of appendix 1) while the contrary is observed with the procedure presented in Appendix C.

- Replacement of subjects during the study would not complicate the statistical model and the analysis if they are dosed at the same time and if they were randomized appropriately. The model and the analysis should remain the same in this case. The complication due to a potential group effect comes into play when the subjects are divided into groups and dosed at different times, or similarly if additional subjects are later dosed to increase the power of the study. Given that replacement of subjects are to be used and specified in the protocol, a different issue often raises: “should we replace subjects who completed, say, 2 out of 3 periods with replacement subjects who completed all 3 periods”. It is generally accepted that replacement of subjects who completed the study over the original subjects who did not, bias our results, even if a balance in terms of sequence and period is maintained.

VI. B. 1. b. Statistical analysis, Data Analysis, Average bioequivalence, Replicated crossover designs

"Linear mixed-effects model procedures, available in PROC MIXED ... should be used for the analysis of replicated crossover studies for average BE."

- When the design is balanced on period (i.e. no missing observations toward period), PROC GLM along with Least squares estimations (or equivalently Methods of Moments) can also be used with a model including period, formulation and sequence as fixed effects and subject nested in sequence and formulation*subject nested in sequence as random effects; the latter term might be removed if not significant.

"Appendix E includes an example of SAS program statements"

- The proposed SAS program was tested on a data set where no subject*form interaction was observed ($\sigma^2_D=0$). Type=UN, type=CSH and the GLM approach were used.

The three structures in PROC MIXED gave the following note in the SAS log: "Estimate G matrix is not positive definite", suggesting that the variance component should be reduced. It is reflected in GLM by a non significant subject*form interaction. The results using FA0(2), UN, CSH and GLM are presented in appendix 2. A dramatic change in the denominator degrees of freedom to test the formulation effect and also to build the confidence interval is observed, depending on the variance-covariance structure. The correct degrees of freedom to use would appear to be 31 but not 99.9 as proposed in FA0(2) or CSH using the Satterthwait correction for degrees of freedom. Thus, concerns should be raised when using replicate designs about how to model such data appropriately.

VI. B. 1. d. Statistical analysis, Data Analysis, Average bioequivalence, Parallel designs

- The following statement does not appear valid: "As in the analysis for replicated designs, equal variances should not be assumed". To perform a classical ANOVA, one of the assumption is equality of variances.

The same comment applies for section VI. B. 2. d: "The method for the upper confidence bound should be modified ... and to allow for unequal variances."

VI. B. 3. Statistical analysis, Data Analysis, Individual bioequivalence

“ For this purpose, we recommend the MM approach... The restricted maximum likelihood (REML) may be useful to estimate mean differences and variances when subjects with some missing data are included in the statistical analysis.”

- The MM approach is recommended for IBE whereas a linear mixed-effects model (REML method) is recommended for ABE using replicated crossover studies. Two different methods are recommended for the same 4-period, 2-sequence replicated design, depending of the criteria used to evaluate bioequivalence. The same method should be recommended since the estimations of the different effects in a model are not “criteria dependent” but “design dependent”.
- When using the method of moments (MM), Appendix H gives the methodology to derive the confidence interval for a four-period replicated design. However, when missing values are present in the dataset, eluding the use of MM, the derivation of a confidence interval might be problematic. We are concerned that the methodology used in Appendix H will not be acceptable if the estimation of variances are made with REML method. This may represent a real problem, because dropouts are expected in replicated designs and REML method might elude the use of a parametric confidence interval.

Appendix G, Variance estimation

“... in addition, the MM approaches have not yet been adapted to models that allow assessment of carryover effects.”

- In Kimanani and Potvin³, the MM have been adapted using Least squares method (LSE) and PROC GLM to derive the estimation of variances. LSE allows the assessment of carryover effects.

APPENDIX 1

Table 1. Individual bioequivalence
Number of subjects⁺ to achieve a power of 80% with $\sigma_D=0.10$

$\sigma_{WR}^2 = \sigma_{WT}^2$	θ_{15}^{**}	
	$\mu_T/\mu_R=1.00$	$\mu_T/\mu_R=1.05$
0.20	42	48
0.25	36	38
0.30	32	34
0.35	32	32
0.40	30	32
0.55	30	30
0.55	28	30
≥ 0.60	28	28

⁺ N such as : $1 - \beta = P_f \left(\frac{(\Delta + 1) F_{\alpha, v, dfer}}{\theta_{15} + 1} \right)_{v, dfer}$ where Δ is the regulatory limit and $P_f(q)_{v, dfer}$ is the probability value of being smaller than the qth quantile of an F distribution with v, dfer degrees of freedom as defined in ³.

⁺⁺ Weighted version of the FDA metric³ with an adjusted regulatory limit of 1.75.

Table 2. Individual bioequivalence
Power (%) to conclude BE with $\sigma_D=0.10$ and N=24

$\sigma_{WR}^2 = \sigma_{WT}^2$	$\mu_T/\mu_R=1.00$			$\mu_T/\mu_R=1.05$		
	θ_{15}^f	θ_{15}^s	θ_{11}^s	θ_{15}^f	θ_{15}^s	θ_{11}^s
0.20	58	57	62	53	53	57
0.30	68	70	71	66	65	68
0.40	72	71	71	70	72	72

θ_{15}^f is the power of the weighted version, calculated using the parametric formula mentioned above (see ⁺ from the previous table).

θ_{15}^s and θ_{11}^s are the estimated power of the weighted and unweighted versions, respectively, using 1000 simulations for each parameters combination and a RTTR, TRRT 4-period 2-sequence replicated crossover design.

Appendix 2

1

The MIXED Procedure

Class Level Information

Class	Levels	Values
SUBJ	33	1 2 3 5 6 7 8 9 10 11 12 13 16 17 18 19 20 22 23 24 25 26 28 29 30 31 32 33 34 35 36 37 38
PERIOD	4	1 2 3 4
FORM	2	A B
SEQUENCE	2	1 2

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	138.08181586	
1	4	5.38697955	0.11134654
2	1	5.07222481	0.00113854
3	1	5.06931172	0.00000021
4	1	5.06931119	0.00000000

Convergence criteria met.

G Matrix

Effect	SUBJ	FORM	Row	COL1	COL2
FORM	1	A	1	0.73275877	0.78987975
FORM	1	B	2	0.78987975	0.85145349

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Group	Estimate
FA(1,1)	SUBJ		0.85601330
FA(2,1)	SUBJ		0.92274237
FA(2,2)	SUBJ		-0.00000000
DIAG	SUBJ	FORM A	0.14088305
DIAG	SUBJ	FORM B	0.16377298

Model Fitting Information for LAUCT

Description	Value
Observations	132.0000
Res Log Likelihood	-118.321
Akaike's Information Criterion	-123.321
Schwarz's Bayesian Criterion	-130.412
-2 Res Log Likelihood	236.6418
Null Model LRT Chi-Square	133.0125
Null Model LRT DF	4.0000
Null Model LRT P-Value	0.0000

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
FORM	1	99.9	17.17	0.0001
SEQUENCE	1	28.4	4.05	0.0538

2

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
PERIOD	3	96.7	26.21	0.0001

ESTIMATE Statement Results

Parameter	Estimate	Std Error	DF	t	Pr > t
T-R	0.28571961	0.06895868	99.9	4.14	0.0001

ESTIMATE Statement Results

Alpha	Lower	Upper
0.1	0.1712	0.4002

3

90% CI for Average Bioequivalence LAUCT

RATIO T/R	Lower Limit	Upper Limit	Standard Error
1.33072	1.18676	1.49214	0.06895868

The MIXED Procedure

Class Level Information

Class	Levels	Values
SUBJ	33	1 2 3 5 6 7 8 9 10 11 12 13 16 17 18 19 20 22 23 24 25 26 29 29 30 31 32 33 34 35 36 37 38
PERIOD	4	1 2 3 4
FORM	2	A B
SEQUENCE	2	1 2

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	138.08181586	
1	2	2.65037391	0.34273892
2	1	2.13780771	0.02862402
3	1	2.10561684	0.00020480
4	1	2.10540016	0.00000001
5	1	2.10540015	0.00000000

Convergence criteria met.

G Matrix

Effect	SUBJ	FORM	Row	COL1	COL2
FORM	1	A	1	0.70971212	0.80248749
FORM	1	B	2	0.80248749	0.82472050

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Group	Estimate
UN(1,1)	SUBJ		0.70971212
UN(2,1)	SUBJ		0.80248749
UN(2,2)	SUBJ		0.82472050
DIAG	SUBJ	FORM A	0.16359432
DIAG	SUBJ	FORM B	0.19004647

Model Fitting Information for LAUCT

Description	Value
Observations	132.0000
Res Log Likelihood	-116.839
Akaike's Information Criterion	-121.839
Schwarz's Bayesian Criterion	-128.930
-2 Res Log Likelihood	233.6779
Null Model LRT Chi-Square	135.9764
Null Model LRT DF	4.0000
Null Model LRT P-Value	0.0000

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
FORM	1	31	25.33	0.0001
SEQUENCE	1	31	4.05	0.0529
PERIOD	3	60.8	22.94	0.0001

ESTIMATE Statement Results

Parameter	Estimate	Std Error	DF	t	Pr > t
T-R	0.28571961	0.05677593	31	5.03	0.0001

ESTIMATE Statement Results

Alpha	Lower	Upper
0.1	0.1895	0.3820

6

90% CI for Average Bioequivalence LAUCT

RATIO T/R	Lower Limit	Upper Limit	Standard Error
1.33072	1.20859	1.46519	0.05677593

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The MIXED Procedure

Class Level Information

Class	Levels	Values
SUBJ	33	1 2 3 5 6 7 8 9 10 11 12 13
		16 17 18 19 20 22 23 24 25 26
		28 29 30 31 32 33 34 35 36 37
		38
PERIOD	4	1 2 3 4
FORM	2	A B
SEQUENCE	2	1 2

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	138.08181586	
1	3	44.69932811	89.26115315
2	3	11.64284777	0.65503198
3	1	6.83259948	0.36645616
4	1	5.32132989	0.08138459
5	1	5.07905631	0.00371410
6	1	5.06933307	0.00000862
7	1	5.06931119	0.00000000

Convergence criteria met.

G Matrix

Effect	SUBJ	FORM	Row	COL1	COL2
FORM	1	A	1	0.73275662	0.78987744
FORM	1	B	2	0.78987744	0.85145101

Covariance Parameter Estimates (REML)

Cov Parm	Subject	Group	Estimate
Var(1)	SUBJ		0.73275662
Var(2)	SUBJ		0.85145101
CSH	SUBJ		1.00000000
DIAG	SUBJ	FORM A	0.14088305
DIAG	SUBJ	FORM B	0.16377298

Model Fitting Information for LAUCT

Description	Value
Observations	132.0000
Res Log Likelihood	-118.321
Akaike's Information Criterion	-123.321
Schwarz's Bayesian Criterion	-130.412
-2 Res Log Likelihood	236.6418
Null Model LRT Chi-Square	133.0125
Null Model LRT DF	4.0000
Null Model LRT P-Value	0.0000

8

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
FORM	1	99.9	17.17	0.0001
SEQUENCE	1	28.4	4.05	0.0538
PERIOD	3	96.7	26.21	0.0001

ESTIMATE Statement Results

Parameter	Estimate	Std Error	DF	t	Pr > t
T-R	0.28571961	0.06895868	99.9	4.14	0.0001

ESTIMATE Statement Results

Alpha	Lower	Upper
0.1	0.1712	0.4002

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90% CI for Average Bioequivalence LAUCT

RATIO T/R	Lower Limit	Upper Limit	Standard Error
1.33072	1.18676	1.49214	0.06895868

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
SEQUENCE	2	1 2
PERIOD	4	1 2 3 4
SUBJ	33	1 2 3 5 6 7 8 9 10 11 12 13 16 17 18 19 20 22 23 24 25 26 28 29 30 31 32 33 34 35 36 37 38
FORM	2	A B

Number of observations in data set = 132

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General Linear Models Procedure

Dependent Variable: LAUCT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	67	134.450125	2.006718	11.35	0.0001
Error	64	11.313660	0.176776		
Corrected Total	131	145.763786			

R-Square	C.V.	Root MSE	LAUCT Mean
0.922384	743.8930	0.42045	0.05652

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SEQUENCE	1	13.429426	13.429426	75.97	0.0001

PERIOD	3	12.231508	4.077169	23.06	0.0001
FORM	1	2.691504	2.691504	15.23	0.0002
SUBJ(SEQUENCE)	31	102.803068	3.316228	18.76	0.0001
SUBJ*FORM(SEQUENCE)	31	3.294619	0.106278	0.60	0.9389

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SEQUENCE	1	13.429426	13.429426	75.97	0.0001
PERIOD	2	12.003271	6.001636	33.95	0.0001
FORM	1	2.691504	2.691504	15.23	0.0002
SUBJ(SEQUENCE)	31	102.803068	3.316228	18.76	0.0001
SUBJ*FORM(SEQUENCE)	31	3.294619	0.106278	0.60	0.9389

General Linear Models Procedure
Least Squares Means

Standard Errors and Probabilities calculated using the Type III MS for
SUBJ*FORM(SEQUENCE) as an Error term

FORM	LAUCT LSMEAN	Pr > T H0: LSMEAN1=LSMEAN2
A	0.20904968	0.0001
B	-0.07666993	

General Linear Models Procedure

Source	Type III Expected Mean Square
SEQUENCE	Var(Error) + 2 Var(SUBJ*FORM(SEQUENCE)) + 4 Var(SUBJ(SEQUENCE)) + Q(SEQUENCE)
PERIOD	Var(Error) + Q(PERIOD)
FORM	Var(Error) + 2 Var(SUBJ*FORM(SEQUENCE)) + Q(FORM)
SUBJ(SEQUENCE)	Var(Error) + 2 Var(SUBJ*FORM(SEQUENCE)) + 4 Var(SUBJ(SEQUENCE))
SUBJ*FORM(SEQUENCE)	Var(Error) + 2 Var(SUBJ*FORM(SEQUENCE))

General Linear Models Procedure
Tests of Hypotheses for Mixed Model Analysis of Variance

Dependent Variable: LAUCT

Source: SEQUENCE		Error: MS(SUBJ(SEQUENCE))		Denominator	Denominator	F Value	Pr > F
DF	Type III MS	DF	MS	DF	MS		
1	13.429425963	31	3.3162279902			4.0496	0.0529

Source: PERIOD		Error: MS(Error)		Denominator	Denominator	F Value	Pr > F
DF	Type III MS	DF	MS	DF	MS		
2	6.0016356655	64	0.1767759443			33.9505	0.0001

Source: FORM

Error: MS(SUBJ*FORM(SEQUENCE))

DF	Type III MS	Denominator	Denominator	F Value	Pr > F
		DF	MS		
1	2.69150414	31	0.1062780341	25.3251	0.0001

Source: SUBJ(SEQUENCE)

Error: MS(SUBJ*FORM(SEQUENCE))

DF	Type III MS	Denominator	Denominator	F Value	Pr > F
		DF	MS		
31	3.3162279902	31	0.1062780341	31.2033	0.0001

Source: SUBJ*FORM(SEQUENCE)

Error: MS(Error)

DF	Type III MS	Denominator	Denominator	F Value	Pr > F
		DF	MS		
31	0.1062780341	64	0.1767759443	0.6012	0.9389

90% CI for Average Bioequivalence LAUCT

RATIO T/R	Lower Limit	Upper Limit	Standard Error	Power %	CV %
1.33072	1.20859	1.46519	0.056776	96.5981	43.9731

1. Jones B and Kenward MG. (1989) Design and Analysis of cross-over trials, Chapman and Hall, London New York (pp 178-180).
2. Kimanani (1999). Definition of individual bioequivalence: Occasion-to-occasion versus mean switchability; *Statistics in Medicine*, (in press).
3. Kimanani and Potvin (1997). A parametric confidence interval for individual bioequivalence; *Journal of Pharmacokinetics and Biopharmaceutics*, 25(5):595-614.