EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

1 16 July 2010
2 EMEA/CVMP/016/00-Rev.2-CONSULTATION
3 Committee for Medicinal Products for Veterinary Use (CVMP)

4 Guideline on the conduct of bioequivalence studies for 5 veterinary medicinal products

6 Draft

Draft Agreed by Efficacy Working Party	February 2009
Draft Agreed by Quality Working Party	February 2009
Draft Agreed by Safety Working Party	February 2009
Adoption by CVMP for release for consultation	**11 March 2009**
End of consultation (deadline for comments)	30 September 2009
Revised draft GL agreed by Efficacy Working Party	May 2010
Revised draft GL agreed by Quality Working Party	May 2010
Revised draft GL agreed by Safety Working Party	May 2010
Revised draft GL adopted by CVMP for release for consultation	***14 July 2010***
End of consultation (deadline for comments)	*31 October 2010*

7
8 This guideline replaces *"Guideline on the conduct of bioequivalence studies for*
9 *veterinary medicinal products" (EMEA/CVMP/016/00-corr*)

10

Comments should be provided using this template. The completed comments form should be sent to vet-guidelines@ema.europa.eu or Fax +44 20 7418 8447

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Keywords	*Bioequivalence, biowaiver, generics, veterinary medicines*

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13 # Guideline on the conduct of bioequivalence studies for
14 veterinary medicinal products

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72 # Executive summary

73 It is the objective of this guidance to specify requirements for the design, conduct, and evaluation of
74 bioequivalence studies for pharmaceutical forms with systemic action. In addition, guidance is given on
75 how *in-vitro* data in specific cases may be used to allow bridging of safety and efficacy data.

76 # 1. Introduction (background)

77 For two products, pharmacokinetic equivalence (i.e. bioequivalence) is established if the rate and
78 extent of absorption of the active substance investigated under identical and appropriate experimental
79 conditions only differ within acceptable predefined limits. Rate and extent of absorption are typically
80 estimated by C_{max} (peak concentration) and AUC (total exposure over time), respectively, in plasma.

81 Bioequivalence studies are often part of applications for generic veterinary medicinal products to allow
82 bridging of safety and efficacy data associated with the reference medicinal product. Other types of
83 applications may also require demonstration of bioequivalence or other comparative pharmacokinetic
84 data (see section 4).

85 # 2. Scope

86 The aim of this guideline is to provide guidance regarding study design, conduct and evaluation of
87 bioequivalence studies for pharmaceutical forms with systemic action and *in-vitro* dissolution tests. In
88 addition, recommendations are given on when *in-vivo* studies are mandatory and when *in-vitro* data
89 are likely to be sufficient.

90 If bioequivalence cannot be demonstrated using pharmacokinetic parameters as endpoints,
91 pharmacodynamic or clinical endpoints may be used, in exceptional circumstances, to demonstrate
92 similar efficacy and safety. However, this situation is outside the scope of this guideline and the reader
93 is referred to therapeutic area specific guidelines where available.

94 Recommendations for modified release products are given in this guideline as there are specific issues
95 to be addressed for these products.

96 The recommendations given for bioequivalence studies in this guideline may also be applied to other
97 comparative pharmacokinetic studies.

98 The scope is limited to chemical entities.

99 # 3. Legal basis

100 This document is intended to provide guidance on the conduct of bioequivalence studies for veterinary
101 medicinal products. It should be read in conjunction with Directive 2001/82/EC, as amended.
102 Applicants should also refer to other relevant European and VICH guidelines, including those listed
103 under 'References'.

104 # 4. Situations when bioequivalence may be applicable

105 Bioequivalence data may be pivotal in a number of different situations. In the following text the level
106 of detail differs according to the anticipated need for guidance and some parts, as indicated in the text,
107 are applicable for generic products only.

4.1. Product development prior to first authorisation of a veterinary medicinal product containing a NCE or a known active substance

During development of a product containing a NCE or a known active substance, bioequivalence studies or other comparative pharmacokinetic data may be needed as bridging studies between different formulations e.g. between pivotal and early clinical trial formulations.

For this purpose, bioequivalence within the acceptance limits as defined in this document might not be needed and study designs other than those presented in this document might be found appropriate. For example, where a tolerance study (systemic tolerance to the active substance) is performed with a different formulation, it will be sufficient to show that the rate and extent of absorption from this formulation is at least as high as that for the formulation intended to be marketed.

4.2. Extensions and variations

Approvals of extensions and variations such as alternative pharmaceutical forms, new dosage strengths, new routes of administration or significant changes to manufacturing or composition which may impact on bioavailability often need support of bioequivalence studies. Waivers from bioequivalence studies should always be justified.

4.3. Applications according to Directive 2001/82/EC as amended, Article 13(3)

This type of application refers to situations where the strict definition of a 'Generic veterinary medicinal product' as outlined in Directive 2001/82/EC, Article 13(2)(b) is not met. This includes conditions where bioavailability studies cannot be used to demonstrate bioequivalence (for example where the new product is supra-bioavailable) or where there are changes in the active substance(s), therapeutic indications, strength, pharmaceutical form or route of administration of the generic product compared to the reference product. In most cases comparative pharmacokinetic data are needed as part of such applications.

4.4. Product containing a known substance intended to be a generic according to Directive 2001/82/EC, Article 13(2)(b)

In the case of systemically active substances when reference is made to an approved product in terms of efficacy and safety, bioequivalence to this product should be demonstrated. It should be noted that there are several aspects such as palatability, animal owner's compliance, local tolerance and residue concentrations at the injection site that might differ between products and that are not covered by bioequivalence data. The need to document such aspects might differ between applications and is beyond the scope of this guideline. It should be noted that bioequivalence or waivers cannot be used for extrapolation of withdrawal periods between products having a potential to leave local residues (in particular intramuscular and subcutaneous injectables and dermal applications). In this case, information on the behaviour of residues at the site of administration needs to be assessed before the withdrawal period is extrapolated.

5. The design and conduct of bioequivalence studies

In the following sections, requirements for the design and conduct of bioequivalence studies are formulated. It is assumed that the applicant is familiar with pharmacokinetic principles underlying bioequivalence studies. The design should be based on a reasonable knowledge of the pharmacokinetics of the active substance and the properties of the formulation in question.

5.1. General requirements

Bioequivalence studies should be conducted according to the principles of Good Laboratory Practice (GLP) and/or Good Clinical Practice (GCP), as appropriate.

The study should be designed in such a way that the formulation effect can be distinguished from other effects. If two formulations are compared, a randomised, two-period, two-sequence single dose crossover design is recommended. The treatment periods should be separated by a sufficiently long wash-out period to ensure that drug concentrations are below the lower limit of quantification of the bioanalytical method in all animals at the beginning of the second period and that no physiological effects, such as metabolic enzyme induction, remain from the first period. Normally, at least 5 elimination half-lives are necessary to achieve this.

Under certain circumstances, provided that the study design and the statistical analyses are scientifically sound, alternative well-established designs could be considered such as a parallel design for substances with very long half-life or when growing animals are used. For substances with highly variable disposition where it is difficult to show bioequivalence due to high intra individual variability, different alternative designs have been suggested in literature. It is recommended to ask for scientific advice if it is estimated that a traditional crossover design would not be feasible without the inclusion of a very high number of animals.

Regarding single dose versus multiple dose studies, single dose studies are preferred as the potential to detect a difference in rate of absorption is lower if the active substance is accumulated. Multiple dose designs should be justified and could be considered if, for example, problems of sensitivity of analytical method preclude sufficiently precise plasma concentration measurements after single dose administration.

For the oral route, special attention must be paid to the different factors that are known to affect absorption of the active substance, such as feeding. Feeding may interfere with drug absorption, depending upon the characteristics of the active substance and the formulation. Feeding may also increase the inter- and intra-individual variability in the rate and extent of drug absorption. For these reasons, fasting conditions are recommended in bioequivalence studies for immediate-release oral formulations unless the SPC of the reference product recommends administration only in the fed state, in which case the bioequivalence study should be conducted accordingly. The rationale for conducting a bioequivalence study under fasting or fed conditions should be provided in the protocol. The protocol should describe the diet and feeding regimen that will be used in the study.

5.2. Special considerations for modified release formulations

When bioequivalence studies are used to bridge efficacy and safety data between formulations designed to modify extent, rate or site of absorption, special consideration is needed. In veterinary medicine there are numerous different types of modified release formulations. These could be for oral use such as prolonged release tablets for companion animals or intraruminal boluses. Many modified release formulations are topically applied, such as spot-ons and pour-ons which are absorbed through the skin, or they may be prolonged release injectable formulations. In most cases such products are intended for single dose use. If so, single dose bioequivalence data are normally sufficient to demonstrate similarity between products. For prolonged release formulations intended for repeated dosing where the aim of the modification is to reduce fluctuations during steady state or to reduce the frequency of administration, demonstration of bioequivalence should be based on multiple dose studies if there is accumulation between doses. In such cases, C_{min} is an important parameter to consider, in addition to C_{max} and AUC. If there is no or negligible accumulation, single dose bioequivalence data are normally also sufficient for prolonged release formulations intended for repeated dosing.

For orally administered modified release formulations intended for non ruminants, bioequivalence normally needs to be established under both fed and fasting conditions unless adequately justified.

For pour-ons and spot-ons the main absorption route is through the skin. However, absorption may also occur from the GI-tract if the animals are licking themselves or each other. When conducting bioequivalence studies with products intended for dermal absorption, issues related to possible oral uptake need to be considered.

200 ### *5.3. Special considerations for products for use in medicated feeding stuffs*
201 ### *or drinking water or milk/milk replacer*

202 Premixes and other pharmaceutical forms for in-feed use may be eligible for a biowaiver (see Appendix
203 I).

204 Most veterinary medicinal products, excluding suspensions and emulsions, for use in drinking water or
205 milk are likely to be exempted from the demand of *in-vivo* bioequivalence data (see section 7.1 and
206 Appendix I).

207 In cases where *in-vivo* data cannot be waived, it is recommended to ask for scientific advice regarding
208 the appropriate study design.

209 ### *5.4. Reference and test product*

210 For Article 13(1) and 13(3) marketing authorisation applications reference must be made to the
211 dossier of a reference medicinal product for which a marketing authorisation is or has been granted in
212 the Union on the basis of a complete dossier in accordance with Articles 12 (3), 13a, 13b or 13c of
213 Directive 2001/82/EC, as amended. The product used as the reference product in the bioequivalence
214 study should be part of the global marketing authorisation of the reference medicinal product (as
215 defined in Article 5(1) second subparagraph of Directive 2001/82/EC).

216 If the reference product is approved for several species there will in most cases be a need for multiple
217 bioequivalence studies to cover all species (see section 5.6)

218 For a generic application according to Article 13(1), the test product should be compared with the
219 same pharmaceutical form of a reference product (various immediate-release oral pharmaceutical
220 forms shall be considered to be one and the same, Article 13(2)b of Directive 2001/82/EC). In the case
221 of an application under Article 13(3), the test product may be compared with a pharmaceutical form
222 differing from that of the reference product. In an application for extension of a concerned veterinary
223 medicinal product which has been initially approved under Article 12(3) of Directive 2001/82/EC and
224 when there are several pharmaceutical forms of this product on the market, the formulation used for
225 the initial approval of the concerned product (and which was used in clinical efficacy and safety
226 studies) should be used as the comparator product, unless otherwise justified.

227 Batch control results of the test and reference products should be reported. Unless otherwise justified,
228 the assayed content of the batch used as the test product should not differ by more than 5% from that
229 of the batch used as the reference product determined with the test procedure proposed for routine
230 quality testing of the test product. In order to demonstrate that a representative batch of the reference
231 product with regards to dissolution and assay content has been selected, the applicant should present
232 dissolution profiles and content analysis of advisably more than one single batch of the reference
233 product.

234 The test product used in the study should be representative of the product to be marketed and this
235 should be justified by the applicant.

236 For example, for oral solid forms for systemic action:

237 a) The test product should originate from a batch of at least 1/10 of production scale, unless
238 otherwise justified.

239 b) The production of batches used should provide a high level of assurance that the product and
240 process will be feasible on an industrial scale.

241 c) The characterisation and specification of critical quality attributes of the drug product, such as
242 dissolution, should be established from the test batch, i.e. the clinical batch for which
243 bioequivalence has been demonstrated.

244 d) Samples of the product from additional pilot and / or full scale production batches, submitted to
245 support the application, should be compared with those of the bioequivalence study test batch,

246 and should show similar *in-vitro* dissolution profiles when employing suitable dissolution test
247 conditions.

248 Comparative dissolution profile testing should be undertaken on the first three production
249 batches.

250 If full scale production batches are not available at the time of submission, the applicant should
251 not market a batch until comparative dissolution profile testing has been completed.

252 The results should be provided at a Competent Authority's request or if the dissolution profiles
253 are not similar together with proposed action to be taken.

254 For other immediate release pharmaceutical forms for systemic action, justification of the
255 representative nature of the test batch should be similarly established.

256 *5.5. Animals*

257 The number of test animals must be appropriate for statistical analyses and should be carefully
258 estimated and justified in the protocol. Where the number of animals necessary to demonstrate
259 bioequivalence cannot be precisely estimated, a two-stage approach can be chosen (see section 5.15).

260 Animals used in bioequivalence studies should be clinically healthy representatives of the target
261 population, and preferably from a homogeneous group (age, breed, gender, weight, hormonal and
262 nutritional status, level of production, etc.). However, when it is difficult to achieve homogeneity of all
263 animals within a study it is acceptable to use a non-homogenous stock, provided that the study is of a
264 crossover design as each animal acts as its own control in such studies.

265 In parallel design studies, the treatment groups should be homogeneous and comparable in all known
266 prognostic variables that affect the pharmacokinetics of the active substance e.g. age, weight, gender
267 etc. (if relevant). This is an essential pre-requisite to give validity to the study results.

268 *5.6. Species to be studied*

269 The test animals should be of the target species. Where a product is intended for more than one
270 species, bioequivalence studies should normally be performed in each target animal species.
271 Extrapolation of results from a major species in which bioequivalence has been established to minor
272 species could be acceptable if justified based on scientific information to demonstrate similarity in the
273 anatomy and physiology (such as pH in the GI tract, gastric volume and GI-tract transit time in the
274 case of oral formulations, injection site anatomy and physiology in the case of injectable formulations
275 etc.).

276 If bioequivalence is established based on a study where widened acceptance limits for C_{max} have been
277 accepted (see section 5.15), data cannot be extrapolated to any other species.

278 *5.7. Route of administration*

279 For applications for generic products, the route of administration should always be the same for test
280 and reference products. When the generic product is intended for more than one route of
281 administration (e.g. both intramuscular and subcutaneous administration), all different routes should
282 be tested unless justified as biowaivers.

283 *5.8. Strength to be tested*

284 If an application concerns several strengths of the active substance, a bioequivalence study
285 investigating only one strength may be acceptable (see section 7.2). If the strength of the test product
286 differs from that of the reference product and this precludes equal doses in the two treatment groups,
287 it is recommended to use different doses and then dose normalise (i.e. to divide AUC and C_{max} with the

288 amount administered) the pharmacokinetic parameters. Prerequisites for dose normalisation are that it
289 was prospectively defined in the protocol and that there is linear pharmacokinetics for the active
290 substance. Care should be taken to ensure that solid oral pharmaceutical forms are not manipulated in
291 a way that could bias the bioequivalence study. In general, all sorts of manipulation such as grinding
292 or filing in order to achieve equal doses should be avoided. Tablets intended to be divided may be
293 divided along their score lines but not into smaller pieces.

294 The same strength should be administered to all animals throughout the entire study independent of
295 their bodyweight unless the animals differ substantially in body size (see section 5.9).

296 *5.9. Dose to be tested*

297 Bioequivalence studies may be performed with any approved dose. However, it is acknowledged that
298 for some animal species e.g. the dog, it could be difficult to find animals suitable for investigation of
299 high strength solid pharmaceutical forms. In this case overdose studies might be considered if
300 tolerated.

301 Most products have a single approved dose adjusted for body size which is expressed as e.g. mg/kg
302 bodyweight. Thus, exact dosing can only be achieved for pharmaceutical forms that allow an indefinite
303 number of dose levels (such as an oral suspension). For all solid pharmaceutical forms the amount to
304 be administered will depend on the different strengths available and the exact dose per kg bodyweight
305 might therefore vary somewhat between animals and potentially within animals over time due to
306 change in bodyweight. To limit the amount of bias introduced due to difficulties regarding dose
307 accuracy the following should be considered:

308 a) If there are no tolerance concerns, administration of higher or lower doses than the approved dose
309 may be acceptable acknowledging the fact that there might not be suitable strengths available to
310 allow the approved weight-adjusted dose to be administered to all animals included in the study.

311 b) The amount administered should be the same in each individual in all periods regardless of
312 changes in body weights between study periods, unless the change in body weight is considerable.

313 c) An attempt should be made to minimise differences in weight between the test animals in order to
314 maintain the same dose across study animals.

315 d) When a solid oral pharmaceutical form is compared to a pharmaceutical form that allows an
316 indefinite number of dose levels, the amount administered should (for both formulations) depend
317 on the options available with the solid form.

318 *5.10. Suprabioavailability*

319 If suprabioavailability is found, i.e. if the test product displays an extent of absorption appreciably
320 larger than the reference product following administration of the same dose, the bioequivalence
321 concept could be a useful tool to demonstrate that equivalent AUC and C_{max} are achieved following
322 administration of a lower dose of the test product as compared to the reference. It may then be
323 expected that the two products have similar systemic efficacy and safety although administered at
324 different doses. It should be noted that suprabioavailable products cannot be generics, but rather
325 applications according to Article 13(3) of Directive 2001/82/EC, as amended, or extension applications.

326 *5.11. Analytes to be measured*

327 **Parent compound or metabolites**

328 <u>General recommendations</u>

329 In principle, evaluation of bioequivalence should be based upon measured concentrations of the parent
330 compound. The reason for this is that C_{max} of a parent compound is usually more sensitive to detect
331 differences between formulations in absorption rate than C_{max} of a metabolite.

332 Inactive pro-drugs

333 In the context of this guideline, a parent compound can be considered to be an inactive pro-drug if it
334 has no or very low contribution to clinical efficacy. For inactive pro-drugs, demonstration of
335 bioequivalence for the parent compound is recommended and the active metabolite does not need to
336 be measured. However, some pro-drugs may have low plasma concentrations and be quickly
337 eliminated resulting in difficulties in demonstrating bioequivalence for the parent compound. In this
338 situation it is acceptable to demonstrate bioequivalence for the main active metabolite without
339 measurement of the parent compound.

340 Use of metabolite data as surrogate for active parent compound

341 The use of a metabolite as a surrogate for an active parent compound is not encouraged. This can only
342 be considered if the applicant can adequately justify that the sensitivity of the analytical method for
343 measurement of the parent compound cannot be improved. Due to recent developments in
344 bioanalytical methodology it is unusual that the parent drug cannot be measured accurately and
345 precisely. Hence, the use of a metabolite as a surrogate for the active parent compound is expected to
346 be accepted only in exceptional cases. When using metabolite data as a substitute for the active parent
347 drug concentrations, the applicant should present any available data supporting the view that the
348 metabolite exposure will reflect the parent drug.

349 **Enantiomers**

350 The use of achiral bioanalytical methods is generally acceptable. However, the individual enantiomers
351 should be measured when all the following conditions are met:

352 a) the enantiomers exhibit different pharmacokinetics

353 b) the enantiomers exhibit pronounced differences in pharmacodynamics

354 c) the exposure (AUC) ratio of enantiomers is modified by a difference in the rate of absorption

355 If one enantiomer is pharmacologically active and the other is inactive or has a low contribution to
356 activity, it is sufficient to demonstrate bioequivalence for the active enantiomer. Further, the use of
357 achiral bioanalytical methods is possible when both products contain the same single enantiomer and
358 there is no inter-conversion *in vivo*.

359 **Endogenous substances**

360 If the substance being studied is endogenous, the calculation of pharmacokinetic parameters should be
361 performed using baseline correction so that the calculated pharmacokinetic parameters refer to the
362 additional concentrations provided by the treatment.

363 The exact method for baseline correction should be pre-specified and justified in the study protocol. In
364 general, the standard subtractive baseline correction method, meaning either subtraction of the mean
365 of individual endogenous pre-dose concentrations or subtraction of the individual endogenous pre-dose
366 AUC, is preferred. In rare cases where substantial increases over baseline endogenous levels are seen,
367 baseline correction may not be needed.

368 In bioequivalence studies with endogenous substances, it cannot be directly assessed whether carry-
369 over has occurred, so extra care should be taken to ensure that the washout period is of an adequate
370 duration.

371 ## *5.12. Sampling Time Considerations*

372 A sufficient number of samples to adequately describe the plasma concentration-time profile should be
373 collected. The sampling schedule should include frequent sampling around the predicted t_{max} to provide
374 a reliable estimate of peak exposure. The sampling schedule should be planned to avoid C_{max} being the
375 first point of a concentration time curve. It should also cover the plasma concentration-time curve for
376 long enough to provide a reliable estimate of the extent of exposure which is achieved if AUC_t is at

least 80% of AUC_{∞}. At least three to four samples are needed during the terminal log-linear phase in order to reliably estimate the terminal rate constant (which is needed for a reliable estimate of AUC_{∞}).

For active substances with a long half-life, relative bioavailability can be adequately estimated using truncated AUC as long as the absorption phase has been completed during the applied sample collection period.

In multiple-dose studies, the pre-dose sample should be taken immediately before dosing and the last sample is recommended to be taken as close as possible to the end of the dosage interval to ensure an accurate determination of AUC . Sampling should also be performed to show that steady state conditions are reached (i.e. trough concentrations during the loading period should be sampled until C_{min} is stable).

For endogenous substances, the sampling schedule should allow characterisation of the endogenous baseline profile for each animal in each period. Often, a baseline is determined from 2-3 samples taken before the drug products are administered.

5.13. Parameters

Actual time of sampling should be used in the estimation of the pharmacokinetic parameters.

In single dose studies AUC_t, AUC_{∞}, C_{max} and t_{max} should be determined and bioequivalence should be based on AUC_t and C_{max}.

In steady state studies AUC_{τ}, $C_{max,ss,}$ $C_{min,ss,}$ and $t_{max,ss}$ should be determined and bioequivalence should be based on AUC_{τ}, $C_{max,ss}$ and $C_{min,ss.}$.

Additional parameters that may be relevant to report from studies include $_{z}$, $t_{1/2}$ and t_{lag}. Parameters may only be dose normalised in special cases (see section 5.8).

Non-compartmental methods should be used for determination of pharmacokinetic parameters in bioequivalence studies. The use of compartmental methods for the estimation of parameters is not acceptable.

5.14. Chemical analysis

The analytical methods used in bioequivalence studies must comply with standard criteria of validation as given in the VICH guideline GL 1 Validation of analytical procedures: definition and terminology (CVMP/VICH/97/076).

The bioanalytical part of bioequivalence trials should be conducted according to the principles of GLP. However, as such studies fall outside the formal scope of GLP, the sites conducting the studies are not required to be certified as part of the GLP compliance certification scheme.

The bioanalytical methods used must be well characterised, fully validated and documented to yield reliable results that can be satisfactorily interpreted.

The main characteristics of a bioanalytical method which are essential to ensure the acceptability of the performance and the reliability of analytical results are: selectivity, lower limit of quantitation, the response function (calibration curve performance), accuracy, precision and stability.

The lower limit of quantitation should be 1/20 of Cmax or lower, as pre-dose concentrations should be detectable at 5% of Cmax or lower (5.15, Reasons for exclusion).

Reanalysis of study samples should be predefined in the study protocol (and/or SOP) before the actual start of the analysis of the samples. Normally reanalysis of subject samples because of a pharmacokinetic reason is not acceptable. This is especially important for bioequivalence studies, as this may bias the outcome of such a study.

Analysis of samples should be conducted without information on treatment.

420 ## 5.15. Evaluation

421 In bioequivalence studies, the pharmacokinetic parameters should in general not be dose normalised.
422 However, it may be justified in exceptional cases where a reference batch with an assay content
423 differing less than 5% from the test product cannot be found (see section 5.4). In such cases, this
424 should be pre-specified in the protocol and justified by inclusion of the results from the assay of the
425 test and reference products in the protocol if relevant.
426
427 Dose normalisation could also be accepted in cases where the strengths of the test product differ from
428 those of the reference product and this precludes equal doses (see section 5.8).

429 **Animal accountability**

430 Ideally, all treated animals should be included in the statistical analysis. However, animals in a
431 crossover trial who do not provide data for both the test and reference products (or who fail to provide
432 data for the single period in a parallel group trial) should not be included.

433 **Reasons for exclusion**

434 Unbiased assessment of the results from randomised studies requires that all animals are observed
435 and treated according to the same rules. These rules should be independent from treatment or
436 outcome. In consequence, the decision to exclude an animal from the statistical analysis must be
437 made before bioanalysis.

438 Exclusion of data cannot be accepted on the basis of statistical analysis or for pharmacokinetic reasons
439 alone, because it is impossible to distinguish the formulation effects from other effects influencing the
440 pharmacokinetics.

441 The exceptions to this are:

442 a) An animal with lack of any measurable concentrations or only very low plasma concentrations for
443 the reference medicinal product. An animal is considered to have very low plasma concentrations if
444 its AUC is less than 5% of the reference medicinal product geometric mean AUC (which should be
445 calculated without inclusion of data from the outlying animal). The exclusion of data for this reason
446 will only be accepted in exceptional cases and may question the validity of the trial.

447 b) Animals for whom the pre-dose concentration is greater than 5 percent of the C_{max} value for the
448 animal in that period. In such cases, the statistical analysis should be performed with the data
449 from that animal for that period excluded. In a 2-period trial this will result in the animal being
450 removed from the analysis. This approach does not apply to endogenous drugs.

451 **Parameters to be analysed and acceptance limits**

452 The parameters to be analysed are $AUC_{t,}$ C_{max} and C_{min} (if applicable). A statistical evaluation of t_{max} is
453 not required. For AUC, the ratio of the two treatment means should be entirely contained within the
454 limits 80% to 125%. The acceptance limits for C_{max} and C_{min} should also generally be within 80% to
455 125%. However, as these parameters may exhibit a greater intra-individual variability, a maximal
456 widening of the limits to 70% to 143% could in rare cases be acceptable if it has been prospectively
457 defined in the protocol together with a justification from efficacy and safety perspectives. Valid data
458 would be, for example, data on PK/PD relationships for efficacy and safety which demonstrate that the
459 proposed wider range does not affect efficacy and safety in a clinically significant way. If PK/PD data
460 are not available, persuasive clinical data may still be used for the same purpose. With regard to
461 antimicrobials and antiparasitic products, risks for resistance development should also be considered
462 when defining acceptance limits.

463 *Post hoc* justifications of wider acceptance limits are not acceptable for any parameter.

464 If bioequivalence data are used to substantiate an extrapolation of a withdrawal period between
465 formulations, the entire 90% confidence interval for the ratio should be within the 80% to 125%
466 acceptance limits for both AUC and C_{max}. If broader limits are used, then residue data to confirm the
467 withdrawal period are required unless their absence can be justified (see also section 4.4).

468 **Statistical analysis**

469 The assessment of bioequivalence is based upon 90% confidence intervals for the ratio of the
470 population geometric means (test/reference) for the parameters under consideration. This method is
471 equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance
472 level.

473 The pharmacokinetic parameters under consideration should be analysed using ANOVA. The data
474 should be transformed prior to analysis using a logarithmic transformation. A confidence interval for
475 the difference between formulations on the log-transformed scale is obtained from the ANOVA model.
476 This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio
477 on the original scale. A non-parametric analysis is not acceptable.

478 The precise model to be used for the analysis should be pre-specified in the protocol. The statistical
479 analysis should take into account sources of variation that can be reasonably assumed to have an
480 effect on the response variable. The terms to be used in the ANOVA model are usually sequence,
481 animal within sequence, period and formulation. Fixed effects, rather than random effects, should be
482 used for all terms.

483 **Two-stage design**

484 It is acceptable to use a two-stage approach when attempting to demonstrate bioequivalence. An
485 initial group of animals can be treated and their data analysed. If bioequivalence has not been
486 demonstrated an additional group can be recruited and the results from both groups combined in a
487 final analysis. If this approach is adopted appropriate steps must be taken to preserve the overall type
488 I error of the experiment and the stopping criteria should be clearly defined prior to the study. The
489 analysis of the first stage data should be treated as an interim analysis and both analyses conducted at
490 adjusted significance levels (with the confidence intervals accordingly using an adjusted coverage
491 probability which will be higher than 90%). For example, using 94.12% confidence intervals for both
492 the analysis of stage 1 and the combined data from stage 1 and stage 2 would be acceptable, but
493 there are many acceptable alternatives and the choice of how much alpha to spend at the interim
494 analysis is at the company's discretion. The plan to use a two-stage approach must be pre-specified in
495 the protocol along with the adjusted significance levels to be used for each of the analyses.

496 When analysing the combined data from the two stages, a term for stage should be included in the
497 ANOVA model.

498 **Presentation of data**

499 All individual concentration data and pharmacokinetic parameters should be listed by formulation
500 together with summary statistics such as geometric mean, median, arithmetic mean, standard
501 deviation, coefficient of variation, minimum and maximum. Individual plasma concentration/time
502 curves should be presented in linear/linear and log/linear scale. The method used to derive the
503 pharmacokinetic parameters from the raw data should be specified. The number of points of the
504 terminal log-linear phase used to estimate the terminal rate constant (which is needed for a reliable
505 estimate of AUC_∞) should be specified.

506 For the pharmacokinetic parameters that were subject to statistical analysis, the point estimate and
507 90% confidence interval for the ratio of the test and reference products should be presented.

508 For single dose studies, the percentage of AUC_∞ that is covered by AUC_t should be reported for each
509 animal in each period.

510 The ANOVA tables, including the appropriate statistical tests of all effects in the model, should be
511 submitted. For the normal two-period, two-sequence crossover design, the presentation should include
512 a 2x2-table that presents for each sequence (in rows) and each period (in columns) means, standard
513 deviations and number of observations for the observations in the respective period of a sequence. In
514 addition, tests for difference and the respective confidence intervals for the treatment effect, the
515 period effect, and the sequence effect should be reported as descriptive data.

516 The report should be sufficiently detailed to enable the pharmacokinetics and the statistical analysis to
517 be repeated, e.g. data on actual time of blood sampling after dose, drug concentrations and the values

518 of the pharmacokinetic parameters for each animal in each period and the randomisation scheme
519 should be provided.

520 Drop-out and withdrawal of animals should be fully documented. If available, concentration data and
521 pharmacokinetic parameters from such animals should be presented in the individual listings, but
522 should not be included in the summary statistics.

523 6. Study report

524 *6.1. Bioequivalence study report*

525 The report of the bioequivalence study should give the complete documentation of its protocol, conduct
526 and evaluation. Although bioequivalence studies are normally conducted to GLP standard, the animal
527 phase of the report should be written in accordance with the structure of VICH GL9.

528 Names and affiliations of the responsible investigator(s), the site of the study and the period of its
529 execution should be stated. Audit certificate(s), if available, should be included in the report.

530 The study report should include evidence that the choice of the reference medicinal product is in
531 accordance with Article 13(1) and Article 13(2) of Directive 2001/82/EC, as amended. This should
532 include the reference product name, strength, pharmaceutical form, batch number, manufacturer,
533 expiry date and country of purchase.

534 The name and composition of the test product(s) used in the study should be provided. The batch size,
535 batch number, manufacturing date and, if possible, the expiry date of the test product should be
536 stated.

537 Certificates of analysis of reference and test batches used in the study should be included in an
538 appendix to the study report.

539 Concentration and pharmacokinetic data and statistical analyses should be presented in the level of
540 detail described above (section 5.15, *Presentation of data*).

541 *6.2. Other data to be included in an application*

542 The applicant should submit a signed statement confirming that the test product has the same
543 quantitative composition and is manufactured by the same process as the one submitted for
544 authorisation. A confirmation as to whether the test product is already scaled-up for production should
545 be submitted. Comparative dissolution profiles (see section 7.2) should be provided.

546 The bioanalytical method should be documented in a pre-study validation report. A bioanalytical report
547 should be provided as well. The bioanalytical report should include a brief description of the
548 bioanalytical method used and the results for all calibration standards and quality control samples. A
549 representative number of chromatograms or other raw data should be provided covering the whole
550 concentration range for all standard and quality control samples as well as the specimens analysed.
551 This should include all chromatograms from at least 20% of the animals with QC samples and
552 calibration standards of the runs including these animals.

553 # 7. Waivers from bioequivalence study requirements for
554 immediate release formulations

555 ## *7.1. Comparisons between formulations*

556 The formulation and the characteristics of the active substance are factors which may affect the
557 requirements regarding support of data from bioequivalence studies. When the test product contains a
558 different salt, ester, ether, isomer, mixture of isomers, complex or derivative of an active substance
559 from the reference medicinal product, bioequivalence should be demonstrated in *in-vivo*
560 bioequivalence studies. However, when the active substance in both test and reference products is
561 identical (or the products contain salts with similar properties as defined in Appendix I, section III), *in-*
562 *vivo* bioequivalence studies may in some situations not be required as described below and in
563 Appendix I.

564 Studies to compare the rate and extent of absorption between two formulations or products containing
565 identical active substances are generally not required if both products fulfil one or more of the
566 following conditions:

567 a) The product is to be administered solely as an aqueous intravenous solution containing the same
568 active substance as the currently approved product. However, if any excipients interact with the
569 active substance (e.g. complex formation), or otherwise affect the disposition of the active
570 substance, a bioequivalence study is required unless both products contain the same excipients in
571 very similar quantity and it can be adequately justified that any difference in quantity does not
572 affect the pharmacokinetics of the active substance.

573 b) In the case of products for intramuscular or subcutaneous administration, and when the product is
574 of the same type of solution (aqueous or oily), contains the same concentration of the active
575 substance and the same excipients in similar amounts as the reference product, bioequivalence
576 studies are not required. Moreover, a bioequivalence study is not required for an aqueous solution
577 for intramuscular or subcutaneous administration which has comparable excipients in similar
578 amounts, if it can be demonstrated that the excipients have no impact on the viscosity.

579 c) If the test product is an aqueous oral solution at time of administration and contains an active
580 substance in the same concentration as an approved reference product presented as an aqueous
581 oral solution at time of administration, bioequivalence studies may be waived if the excipients
582 contained in it do not affect gastrointestinal transit (e.g. sorbitol, mannitol, etc.), absorption (e.g.
583 surfactants or excipients that may affect transport proteins), solubility (e.g. co-solvents) or *in-vivo*
584 stability of the active substance. Any differences in the amount of excipients should be justified by
585 reference to other data, otherwise an *in-vivo* bioequivalence study will be required. The same
586 requirements for similarities in excipients apply for oral solutions as for biowaivers according to the
587 relevant criteria (see Appendix I, section IV.2).

588 d) The products are classified as biowaivers in accordance with principles underlying the BCS (see
589 Appendix I).

590 e) The product is intended to be a gas for inhalation at the time of administration.

591 f) The product is a reformulated product by the original manufacturer that is identical to the original
592 product except for small amounts of colouring agents, flavouring agents or preservatives, which
593 are recognised as having no influence upon bioavailability.

594 ## *7.2. Comparisons between strengths*

595 If an application concerns several strengths of the active substance, a bioequivalence study
596 investigating only one strength may be acceptable provided *in-vitro* equivalence data are presented for
597 additional strengths. A pre-requisite is that all of the following conditions are fulfilled:

598 a) the pharmaceutical products are manufactured by the same manufacturing process,

b) the qualitative composition of the different strengths is the same,

c) the composition of the strengths is quantitatively proportional, i.e. the ratio between the amount of each excipient to the amount of active substance(s) is the same for all strengths (for immediate release products, coating components, capsule shell, colour agents and flavours are not required to follow this rule). If there is some deviation from quantitatively proportional composition, condition c) is still considered fulfilled if conditions i) and ii) **or** i) and iii) below apply to the strength used in the bioequivalence study and the strength(s) for which a waiver is considered:
   i. the amount of the active substance(s) is less than 5 % of the tablet core weight, the weight of the capsule content
   ii. the amounts of the different core excipients or capsule content are the same for the concerned strengths and only the amount of active substance is changed
   iii. the amount of a filler is changed to account for the change in amount of active substance. The amounts of other core excipients or capsule content should be the same for the concerned strengths

d) appropriate *in-vitro* dissolution data should confirm the adequacy of waiving additional *in-vivo* bioequivalence testing

The criteria above apply also to the situation where there are several strengths of a generic immediate release product to be approved. If one of the strengths is found to be bioequivalent with the reference product, *in-vitro* data could be sufficient to document bioequivalence for the other strengths of the generic application. Similarity of *in-vitro* dissolution should be demonstrated at all conditions within the applied product series, i.e. between additional strengths and the strength(s) (i.e. batch(es)) used for bioequivalence testing.

The conditions regarding proportional composition should be fulfilled for all active substances of fixed combinations. When considering the amount of each active substance in a fixed combination the other active substance(s) can be considered as excipients. In the case of bilayer tablets, each layer may be considered independently.

At pH values where sink conditions may not be achievable for all strengths *in-vitro* dissolution may differ between different strengths. However, the comparison with the respective strength of the reference medicinal product should then confirm that this finding is active substance rather than formulation related. In addition, the applicant could show similar profiles at the same dose (e.g. as a possibility two tablets of 5 mg versus one tablet of 10 mg could be compared).

General aspects of *in-vitro* dissolution experiments are briefly outlined in section 8, including basic requirements for use of the similarity factor (f2-test).

8. Dissolution testing

During the development of a veterinary medicinal product a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the active substance. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistency and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, in certain instances a dissolution test can be used to demonstrate bioequivalence. Therefore, dissolution studies can serve several purposes:

a) Testing on product quality

- To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control.
- To be used as a tool in quality control to demonstrate consistency in manufacture.
- To get information on the reference product used in bioavailability/bioequivalence studies and pivotal clinical studies.

b) Bioequivalence surrogate inference

- To demonstrate in certain cases similarity between different formulations of an active substance and the reference medicinal product (biowaivers e.g., variations, formulation changes during development and generic products).

- To investigate batch to batch consistency of the products (test and reference) to be used as basis for the selection of appropriate batches for the *in-vivo* study.

Unless otherwise justified, the specifications for the *in-vitro* dissolution to be used for quality control of the product should be derived from the dissolution profile of the test product batch that was found to be bioequivalent to the reference product. In the event that the results of comparative *in-vitro* dissolution of the biobatches do not reflect bioequivalence as demonstrated *in-vivo* the latter prevails. However, possible reasons for the discrepancy should be addressed and justified.

Test methods should be developed which are product-related and based on general and/or specific pharmacopoeial requirements. If those requirements are shown to be unsatisfactory and/or do not reflect the *in-vivo* dissolution (i.e. biorelevance) alternative methods can be considered when it is justified that these are discriminatory and able to differentiate between batches with acceptable and non-acceptable performance of the product *in-vivo*. Current state-of-the-art information including the interplay of characteristics derived from the BCS classification and the pharmaceutical form must always be considered.

Sampling time points should be sufficient to obtain meaningful dissolution profiles, and at least every 15 minutes. More frequent sampling during the period of greatest change in the dissolution profile is recommended. For rapidly dissolving products, where complete dissolution is within 30 minutes, generation of an adequate profile by sampling at 5- or 10-minute intervals may be necessary.

If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pH-range and the excipients are known not to affect bioavailability. A bioequivalence study may in those situations be waived based on similarity of dissolution profiles which are based on discriminatory testing, provided that the other biowaiver criteria in Appendix I are met. The similarity should be justified by dissolution profiles attained at three different buffers spanning the range of possible physiological pH values for the concerned species (e.g. pH 1.2, 4.5 and 7.5).

In contrast, if an active substance is considered to have a limited or low solubility, the rate limiting step for absorption may be pharmaceutical form dissolution. This is also the case when excipients are controlling the release and subsequent dissolution of the active substance. In these cases a variety of test conditions is recommended and adequate sampling should be performed.

Similarity of dissolution profiles

Dissolution profile similarity testing and any conclusions drawn from the results (e.g. justification for a biowaiver) can be considered valid only if the dissolution profile has been satisfactorily characterised using a sufficient number of time points.

Where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical evaluation.

In case more than 85% is not dissolved at 15 minutes but within 30 minutes, at least three time points are required: the first time point before 15 minutes, the second one at 15 minutes and the third time point when the release is close to 85%.

For modified release products, the advice given in the relevant guidance should be followed.

Dissolution similarity may be determined using the $f2$ statistic as follows:

$$f_2 = 50 \cdot \log \left[\frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} \left[\bar{R}(t) - \bar{T}(t) \right]^2}{n}}} \right]$$

In this equation $f2$ is the similarity factor, n is the number of time points, R(t) is the mean percent drug dissolved of e.g. a reference product, and T(t) is the mean percent drug dissolved of e.g. a test product.

694 The evaluation of the similarity factor is based on the following conditions:

695 ▪ A minimum of three time points (zero excluded).
696 ▪ The time points should be the same for the two formulations
697 ▪ Twelve individual values for every time point for each formulation.
698 ▪ Not more than one mean value of > 85% dissolved for any of the formulations.
699 ▪ The relative standard deviation or coefficient of variation of any product should be less than 20%
700 for the first point and less than 10% from second to last time point.

701 An *f2* value between 50 and 100 suggests that the two dissolution profiles are similar.

702 When the ƒ2 statistic is not suitable, then the similarity may be compared using model-independent or
703 model-dependent methods e.g. by statistical multivariate comparison of the parameters of the Weibull
704 function or the percentage dissolved at different time points.

705 Alternative methods to the ƒ2 statistic to demonstrate dissolution similarity are considered acceptable,
706 if statistically valid and satisfactorily justified

707 The similarity acceptance limits should be pre-defined and justified and not be greater than a 10%
708 difference. In addition, the dissolution variability variance of the test and reference product data
709 should also be similar, however, a lower variability of the test product may be acceptable.

710 Evidence that the statistical software has been validated should also be provided.

711 A clear description and explanation of the steps taken in the application of the procedure should be
712 provided, with appropriate summary tables.

Definitions

ANOVA: Analysis of variance model

BCS: Biopharmaceutics Classification System, see Appendix I

Bioavailability: The fraction of an administered dose that reaches the systemic circulation as intact drug.

Bioequivalence: The similarity between two products that contain the same active substance(s) and shows similar rate and extent of absorption of the drug. In most cases the rate and extent of absorption are expressed as C_{max} and AUC. The aim is to show that two medicinal products are similar to such degree that their systemic effects, with respect to both efficacy and safety, will be essentially the same.

Biowaiver: The possibility of waiving *in-vivo* bioequivalence studies.

Comparative pharmacokinetic studies: Any study which compares the pharmacokinetics between products that contain the same active substance. A bioequivalence study is an example of a comparative pharmacokinetic study.

Dose: Amount of active substance(s), to be given to an animal; it is often expressed in mg/kg body weight.

Immediate release formulations: Formulations showing a release of the active substance(s) which is not deliberately modified by a special formulation design and/or manufacturing method. In the case of a solid pharmaceutical form, the dissolution profile of the active substance depends essentially on its intrinsic properties.

Modified release formulations: Formulations where the rate and/or place of release of the active substance(s) is different from that of a conventional-release pharmaceutical form administered by the same route. This deliberate modification is achieved by a special formulation design and/or manufacturing method. Modified-release pharmaceutical forms include prolonged-release, delayed-release and pulsatile-release pharmaceutical forms.

> **Prolonged-release dosage forms:** Prolonged-release dosage forms are modified-release dosage forms showing a slower release of the active substance(s) than that of a conventional-release dosage form administered by the same route. Prolonged-release is achieved by a special formulation design and/or manufacturing method.
>
> **Delayed-release dosage forms:** Delayed-release dosage forms are modified-release dosage forms showing a release of the active substance(s) which is delayed. Delayed release is achieved by a special formulation design and/or manufacturing method. Delayed-release dosage forms include gastro-resistant preparations.
>
> **Pulsatile-release dosage forms:** Pulsatile-release dosage forms are modified-release dosage forms showing a sequential release of the active substance(s). Sequential release is achieved by a special formulation design and/or manufacturing method.

NCE: New chemical entity

Strength: The amount of active substance(s) included in a certain formulation.

Pharmacokinetic parameters

AUC_t:	Area under the plasma concentration curve from administration to last observed concentration at time t;
AUC_∞:	Area under the plasma concentration curve extrapolated to infinite time;
AUC :	AUC during a dosage interval at steady state;
C_{max}:	Maximum plasma concentration;
$C_{max,ss}$:	Maximum plasma concentration at steady state;
$C_{min,ss,}$	Minimum plasma concentration at steady state;
t_{max}:	Time until C_{max} is reached;
$t_{max,ss}$:	Time until $C_{max,ss}$ is reached;
$t_{1/2}$:	Plasma concentration half-life;
$_z$:	Terminal rate constant;
t_{lag}	Absorption lag time

References (scientific and / or legal)

- Guideline on statistical principles for veterinary clinical trials (CVMP/816/00)
- Guideline for the conduct of pharmacokinetic studies in target animal species (EMEA/CVMP/EWP/133/99)
- Guideline on Fixed Combination Products (EMEA/CVMP/83804/2005)
- Guideline for investigations of chiral substances (EMEA/CVMP/128/95)
- Quality of Modified Release Pharmaceutical forms for Veterinary Use (EMEA/CVMP/680/02)
- Good Clinical Practices VICH GL9

784 # APPENDIX I – BCS-Based Biowaivers

785 ## I. Introduction

786 The BCS (Biopharmaceutics Classification System) based biowaiver approach is intended to reduce the
787 requirements for *in-vivo* bioequivalence studies, i.e. it may represent a surrogate for *in-vivo*
788 bioequivalence. *In-vivo* bioequivalence studies may be exempted if an assumption of equivalence in *in-*
789 *vivo* performance can be justified by satisfactory *in-vitro* data. The concept is applicable to solid and
790 semi-solid immediate release pharmaceutical products for oral administration and systemic action
791 having the same pharmaceutical form.

792 The BCS based approach is mainly based on human data and very few studies to validate this system
793 have been conducted in animals. However, the principles behind the BCS based approach could still be
794 effectively applied in veterinary medicine if possible species differences of relevance are considered.
795 Compared to its application in human medicine, a larger variety of GI-tract pH values has to be
796 considered as well as a variety of gastric/intestinal fluid volumes and transit times. Therefore, the
797 approach presented below represents a summary of requirements to fulfil any "worst case scenario".
798 Of note is that in order to apply the BCS system to animals, the solubility classification has been
799 modified in comparison to that used in humans.

800 The application of BCS-based biowaiver is restricted to highly soluble active substances with known
801 absorption in target animals and which are considered non-critical in terms of efficacy and safety.
802 Specific guidance is provided for biowaivers for BCS Class I substances (high solubility, high
803 permeability) and for Class III substances (high solubility, low permeability). The classification is
804 species specific.

805 The principles may be used to establish bioequivalence in applications for generic medicinal products,
806 extensions of innovator products, variations that require bioequivalence testing, and between early
807 clinical trial products and to-be-marketed products.

808 ## II. Summary Requirements

809 BCS-based biowaivers are applicable for an immediate release formulation if:

810 ▪ the active substance has been proven to exhibit high solubility and complete absorption (BCS-
811 Class I; for details see section III), <u>and</u>
812 ▪ very rapid (more than 85% within 15 minutes) *in-vitro* dissolution characteristics of the test
813 and reference product have been demonstrated considering specific requirements (see section
814 IV.1), <u>and</u>
815 ▪ excipients that might affect bioavailability are qualitatively and quantitatively the same. In
816 general, the use of the same excipients in similar amounts is preferred (see section IV.2).

817 BCS-based biowaivers are also applicable for an immediate release formulation if:

818 ▪ the active substance has been proven to exhibit high solubility and limited absorption (BCS-
819 Class III; for details see Annex section III), <u>and</u>
820 ▪ very rapid (more than 85% within 15 minutes) *in-vitro* dissolution characteristics of the test
821 and reference product have been demonstrated considering specific requirements (see section
822 IV.1), <u>and</u>
823 ▪ excipients that might affect bioavailability are qualitatively and quantitatively the same and
824 other excipients are qualitatively the same and quantitatively very similar (see section IV.2).

825 Generally, the risks of an inappropriate biowaiver decision should be more critically reviewed (e.g. site-
826 specific absorption, risk for transport protein interactions at the absorption site, excipient composition
827 and therapeutic risks) for products containing BCS class III compared to BCS class I drug substances.
828 If there are insufficient data available on such aspects for a certain target animal species, biowaivers
829 cannot be granted.

830 Notably, for species where there are considerable differences between subgroups within the species
831 (e.g. ruminant and pre-ruminant cattle), special consideration is needed to cover all the
832 categories/subspecies of animals.

833 III. Active Substance

834 Generally, sound peer-reviewed literature may be acceptable for known compounds to describe the
835 particular characteristics of the active substance required in this biowaiver concept.

836 A biowaiver may be applicable when the active substance(s) in the test and reference products are
837 identical. A biowaiver may also be applicable if test and reference products contain different salts
838 provided that both belong to BCS-class I (high solubility and complete absorption; see sections III.1
839 and III.2). A biowaiver is not applicable when the test product contains a different ester, ether, isomer,
840 mixture of isomers, complex or derivative of an active substance from that of the reference product,
841 since these differences may lead to different bioavailabilities not deducible by means of experiments
842 used in the BCS-based biowaiver concept.

843 The active substance should not have a 'narrow therapeutic window'. This applies to products where a
844 minor difference in plasma concentration would imply a clinically relevant change in the safety and/or
845 efficacy profile. It is not possible to define a set of criteria to define a narrow therapeutic window and it
846 must be decided on a case by case basis.

847 It is recommended to ask for scientific advice before applying the BCS approach to products containing
848 prodrugs.

849 *III.1 Solubility*

850 The pH-solubility profile of the active substance should be determined and discussed. Since gastric and
851 intestinal fluid volumes differ markedly across animal species, the solubility classification in the context
852 of this guideline is different to the classification applied in human medicine. In order to be eligible for a
853 veterinary biowaiver, the active substance should be at least 'soluble' (> 1 g / 30 ml at 15-25°C),
854 according to the definitions of the Ph.Eur (lower temperatures might be relevant for fish and
855 amphibians). This should be demonstrated within the range of possible physiological pH values for the
856 (sub)species and requires the investigation in at least three buffers spanning this range and in addition
857 at the pKa, if it is within the specified pH range. Replicate determinations at each pH condition may be
858 necessary to achieve an unequivocal solubility classification (e.g. shake-flask method or other justified
859 method). Solution pH should be verified prior and after addition of the active substance to a buffer. A
860 lower solubility cut-off may be accepted, if fully justified.

861 *III.2 Absorption*

862 A drug substance is considered to have complete absorption when the extent of absorption has been
863 determined to be ≥ 85 % in comparison to an intravenous reference dose. Complete absorption is
864 generally related to high permeability.

865 Where relevant data are missing in the target animal (sub)species, the drug substance will not be
866 considered to have complete absorption.

867 IV. Veterinary medicinal Product

868 *IV.1 In-vitro Dissolution*

869 *IV.1.1 General aspects*

870 Investigations relating to the medicinal product should ensure immediate release properties and prove
871 similarity between the investigative products, i.e. test and reference product should have a similar *in-*
872 *vitro* dissolution considering physiologically relevant experimental pH conditions (see section 8 of the

873 guideline). *In-vitro* dissolution should be investigated within the physiological pH range relevant for the
874 target animal (sub)species. Additional investigations may be required at pH values in which the active
875 substance has minimum solubility. The use of any surfactant is not acceptable.

876 Test and reference products should meet requirements as outlined in section 5.4 of the main guideline
877 text. In line with these requirements it is advisable to investigate more than one single batch of the
878 test and reference products.

879 Comparative *in-vitro* dissolution experiments should follow current compendial standards. Hence,
880 thorough description of experimental settings and analytical methods including validation data should
881 be provided. It is recommended to use 12 units of the product for each experiment to enable statistical
882 evaluation. Usual experimental conditions are e.g.:

883 ▪ Apparatus: paddle or basket
884 ▪ Volume of dissolution medium: 900 ml or less
885 ▪ Temperature of the dissolution medium: 37±1 °C
886 ▪ Agitation: paddle apparatus - usually 50 rpm
887 basket apparatus - usually 100 rpm
888 ▪ Sampling schedule: e.g. 10, 15, 20, 30 and 45 min
889 ▪ Buffer: e.g. pH 1-1.2 (usually 0.1 N HCl or Simulated Gastric Fluid (SGF) without enzymes),
890 4.5 and 7.5 (or Simulated Intestinal Fluid (SIF) without enzymes); (pH should be ensured
891 throughout the experiment; Ph.Eur. buffers recommended)
892 ▪ Other conditions: no surfactant; in case of gelatin capsules or tablets with gelatin coatings the
893 use of enzymes may be acceptable.

894 Complete documentation of *in-vitro* dissolution experiments is required including a study protocol,
895 batch information on test and reference batches, detailed experimental conditions, validation of
896 experimental methods, individual and mean results and respective summary statistics.

897 *IV.1.2 Evaluation of in-vitro dissolution results*

898 Veterinary medicinal products are considered to be 'very rapidly' dissolving when more than 85% of
899 the labelled amount is dissolved within 15 minutes. In cases where this is ensured for the test and
900 reference product, the similarity of dissolution profiles may be accepted as demonstrated without any
901 mathematical calculation. Generally comparison at 15 minutes is considered to be an acceptable
902 indicator that complete dissolution is reached before gastric emptying. However, the selection of
903 another appropriate time point can be justified by provision of relevant data demonstrating that the
904 selected timepoint is shorter than the gastric emptying time under fed/fasting conditions for the target
905 (sub)species.

906 ***IV.2 Excipients***

907 Although the impact of excipients in immediate release formulations on bioavailability of highly soluble
908 and completely absorbable active substances (i.e., BCS-Class I) is considered rather unlikely it cannot
909 be completely excluded. Therefore, even in the case of Class I drugs it is advisable to use similar
910 amounts of the same excipients in the composition of the test product to those used in the reference
911 product.

912 If a biowaiver is applied for a BCS-class III active substance, excipients have to be qualitatively the
913 same and quantitatively very similar in order to exclude different effects on membrane transporters.

914 As a general rule, for both BCS-class I and III active substances, well-established excipients in usual
915 amounts should be employed and possible interactions affecting drug bioavailability and/or solubility
916 characteristics should be considered and discussed. A description of the function of the excipients is
917 required with a justification of whether the amount of each excipient is within the normal range.
918 Excipients that might affect bioavailability, e.g. sorbitol, mannitol, sodium lauryl sulfate or other
919 surfactants, should be identified as well as their possible impact on

920 ▪ gastrointestinal motility
921 ▪ susceptibility to interactions with the active substance (e.g. complexation)
922 ▪ drug permeability
923 ▪ interaction with membrane transporters

924 Excipients that might affect bioavailability should be qualitatively and quantitatively the same in the
925 test product and the reference product.

926 V. Fixed Combinations

927 BCS-based biowaivers are applicable for immediate release fixed combination products if all active
928 substances in the combination belong to BCS-Class I or III and the excipients fulfil the requirements
929 outlined in section IV.2. Otherwise *in-vivo* bioequivalence testing is required.

930 VI. Biowaivers for pharmaceutical forms for in-feed or in
931 drinking water or milk use

932 *VI.1 Biowaiver for pharmaceutical forms for in-feed use*

933 These products may be treated as immediate release formulations and can be regarded as eligible for
934 a biowaiver if they contain substances that belong to BCS Class I or III.

935 Feed constituents may affect the bioavailability of the active substances administered with feed.
936 However, it is believed that this should not be a factor in considering a biowaiver request since the
937 variability in feed constituents between the test and reference products should not be greater than the
938 natural variations that can occur in the final feed to which the animal will be exposed, whether that
939 feed contains the test product or the reference product. Accordingly, a product for in-feed use which
940 contains insoluble constituents as excipients could also be eligible for a biowaiver, provided the active
941 substance fulfils the BCS criteria.

942 *VI.2 Biowaiver for soluble pharmaceutical forms for in drinking water or milk use*

943 The conceptual basis for granting biowaivers for these soluble pharmaceutical forms is that once a
944 medicinal product is presented in a solution prior to administration, the product's formulation will
945 usually not influence the bioavailability of the active substance. This is because, from a mechanistic
946 perspective, it is believed that the rate-limiting step in systemic drug absorption will be: a) the rate of
947 gastric transit; and b) the permeability of the active substance across the gastrointestinal mucosal
948 membranes. Both of these variables are here formulation-independent.

949 The only exceptions are when the formulation contains substances other than the active substance that
950 could cause a direct pharmacologic effect in the target animal (sub)species (e.g., altered
951 gastrointestinal transit time, membrane permeability, or drug metabolism), or when there is
952 inactivation of the active substance by, for example, a chelating agent.

953 For products to be administered in milk/milk replacer, data to demonstrate solubility and stability in
954 milk should be provided. In order to be exempt from in-vivo studies, the active substance must be
955 demonstrated to be highly soluble in the aqueous milk fraction.