TABLE 6.2
Evaluation of the Need for *In Vivo* Nonclinical Studies (EMA, 2014b, 2015a)

Factors To Be Considered When the Need for *In Vivo* Nonclinical Studies Is Evaluated:

- Presence of potentially relevant quality attributes that have not been detected in the reference product
  - New posttranslational modification structures—atypical glycosylation structures or variants not observed in the reference medicinal product, with particular attention to nonhuman structures (nonhuman linkages, sequences, or sugars)[a]
- Presence of potentially relevant quantitative differences in quality attributes between the biosimilar and the reference product
  - Quantitative differences in posttranslational modification structures (e.g., glycosylation, oxidation, deamidation, truncation)
- Relevant differences in formulation
  - Use of excipients not widely used for biotechnology-derived proteins

[a] Introduction of novel structures, especially when nonhuman, may preclude biosimilarity due to unresolved residual uncertainties on safety.

Step three describes the 3Rs approach to *in vivo* nonclinical studies, reminding us that the type of studies to be performed needs to be critically weighed against the type of additional information needed (PK and/or PD and/or safety issues, with appropriate justification of study design). Testing at one dose level, in one sex, and the omission of the recovery group should be considered wherever possible. The conduct of repeat-dose studies in NHPs is usually not recommended, and studies regarding safety pharmacology, reproduction toxicology, and carcinogenicity are not required for nonclinical testing of biosimilars.

van Meer et al. (2015a) nicely summarized the new European approach to *in vivo* animal studies for biosimilars as "animal studies in the new European Union biosimilar guidance: No longer 'yes, but' but 'no, unless.'"

6.7 WORLDWIDE INTERPRETATION OF EU GUIDELINES/GLOBAL DEVELOPMENT

Currently, there are guidance documents on the development of biosimilars from 26 countries or regions (Krishnan et al., 2015). Following the 2006 EMA guidelines, in 2009 the World Health Organization (WHO) issued a biosimilar guideline and includes a similar approach as initially was done in the EU, that is, requesting animal testing for the nonclinical development step (WHO, 2009). The WHO guidance was accepted by many countries and has been translated to national guidelines over the world, and *in vivo* animal studies are considered mandatory (e.g., Brazil, India, South Korea).

The need to revise the WHO guideline on biosimilars as a consequence of the new European paradigm on requirements has been highlighted by European regulators. Discussions on an international level as to whether a revision of the WHO guideline on biosimilars is feasible are currently ongoing.