Contrary to applications for generic medicinal products, animal studies have traditionally been requested for biosimilars from early on.

While reviewing historical and current regulatory thinking on nonclinical testing of biosimilars and focusing on the evolution in the recommendations in the European Union (EU) guidelines, we aim to clarify the shift in paradigm on the need for *in vivo* testing.

6.2 EUROPE HAS BEEN SETTING THE SCENE

In 2005, the EU was the first region to set up a legal framework and regulatory path for biosimilar development (EC, 2001). The most critical feature of biosimilar development, as explained in the very first European guidance documents (EMA, 2005, 2006), is that it should be demonstrated that the product is (highly) similar to the reference product in terms of quality, safety, and efficacy. It is not to demonstrate *de novo* nonclinical or clinical safety or efficacy because there is already established knowledge from the approved product. Rather, it must be demonstrated that potential subtle differences in quality attributes between the products do not result in clinically relevant differences in safety or efficacy. The importance of the comparability at the level of the pharmaceutical quality, together with the limitations to the extent to which biosimilars could be characterized using physicochemical methods, was already emphasized in the early guidance documents. The final product is often a complex mixture of closely related large-sized molecules, which makes it difficult to provide a complete characterization. The complexity of the final product can be influenced by various factors such as protein aggregation and glycosylation. Today, the methods used to detect differences in quality attributes between products have advanced to a considerable extent. New analytical methods are suitable to detect subtle structural and compositional differences between the biosimilar and the reference product. For a more detailed discussion, see the chapters in this book related to the analytical assessment of biosimilars.

Still, the clinical relevance of the observed subtle quality differences (i.e., the effect on efficacy and safety) is often not clear based on the analytical data alone and further nonclinical and clinical studies may be needed, as reflected in the overarching biosimilar guidelines (EMA, 2014a,b, 2015a). It can be anticipated that in the future control of the biotechnological processes and analytical abilities will have advanced to such a level that the need for additional nonclinical and clinical studies will be further diminished.

6.3 A HISTORICAL REQUEST FOR STANDARD *IN VIVO* STUDIES

Decisions on the marketing authorization of medicinal products, and to an even larger extent approval of clinical trials, are partly based on nonclinical data, including animal studies. Before initiating clinical development, nonclinical studies should be conducted. Nonclinical development of new biologicals requires *in vivo* toxicity studies in relevant animal species, as described in ICH guideline S6 (R1) (ICH, 2011). Typically, this consists of repeated dose toxicity, as well as studies regarding safety pharmacology and reproduction toxicology.