necessarily performed. Note that at the purification step, the following chromatography media or resins are commonly considered: (1) gel filtration, (2) ion exchange, (3) hydrophobic interaction, (4) reversed phase/normal phase, and (5) affinity. Thus, at each step of the manufacturing process, primary performance characteristics should be identified, controlled, and tested for consistency of process control and validation.

Issues of manufacturing and process control in the development of biosimilars are discussed in Chapter 5 of this book.

1.3.4 Similarity in Size and Structure

In practice, sponsors perform various *in vitro* tests such as the assessments of the primary amino acid sequence, charges, and hydrophobic properties to compare the structural aspects of biosimilars with their originator molecules. However, whether *in vitro* tests can be predictive of biological activity *in vivo* is a concern inasmuch as there may be significant differences in biological activity despite similarities in size and structure. Besides, it is difficult to assess biological activity adequately as few animal models can provide the data needed to extrapolate for an accurate and reliable prediction of biological activity in humans. Thus, controlled clinical trials remain often necessary for confirming the similarity between a biosimilar molecule and the originator product.

The FDA has proposed a tiered approach for analytical similarity assessment of CQAs relevant to clinical outcomes at various stages of the manufacturing process. The FDA suggests first classifying the identified CQAs into three tiers depending on their criticality or risk ranking relevant to clinical outcomes. The FDA then recommends using equivalence tests for CQAs in Tier 1 that are considered most relevant to clinical outcomes; a quality range approach for CQAs in Tier 2 that are considered mild to moderate relevant to clinical outcomes; and raw data and graphical comparisons for CQAs in Tier 3 that are considered least relevant to clinical outcomes (see, e.g., Chow et al., 2016). Analytical similarity assessment is discussed in greater detail in Chapter 3 of this book.

1.3.5 Biosimilarity in Biological Activity

Pharmacological or biological activity is an expression describing the beneficial or adverse effects of a drug on living matter. When the drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. A crucial component of biological activity is a substance's toxicity. Activity is generally dosage dependent, and it is not uncommon to have effects ranging from beneficial to adverse for one substance when going from low to high doses. Activity depends critically on the fulfillment of the absorption, distribution, metabolism, and excretion (ADME) criteria.

Note that the EU *Pharmaceutical Review* legislation published on April 30, 2004, amended the EU community code on medicinal products to provide for the approval of biosimilars based on fewer preclinical and clinical data than had been required for the original reference product. The complexity of the protein and knowledge of its structure–function relationships determine the types of information needed to establish similarity.