In vitro assays may include, but are not limited to, biological assays, binding assays, and enzyme kinetics. In vivo assays may include the use of animal models of disease (e.g., models that exhibit a disease state or symptom) to evaluate functional effects on PD markers or efficacy measures. A functional evaluation comparing a proposed product to the reference product that uses these types of assays is also an important part of the foundation that supports a demonstration of biosimilarity, and may be used to scientifically justify a selective and targeted approach to animal and/or clinical testing.

Sponsors can use functional assays to provide additional evidence that the biologic activity and the potency of the proposed product are highly similar to those of the reference product and/or to support a conclusion that there are no clinically meaningful differences between the proposed product and the reference product. Such assays also may be used to provide additional evidence that the MOAs of the two products are the same to the extent that the MOA of the reference product is known. Functional assays can be used to provide additional data to support results from structural analyses, investigate the consequences of observed structural differences, and explore structure–activity relationships. These assays are expected to be comparative so they can provide evidence of similarity or reveal differences in the performance of the proposed product compared to that of the reference product, especially the differences resulting from variations in structure that cannot be detected using current analytical methods. The FDA also recommends that sponsors discuss limitations of the assays they used when interpreting results in their submissions to FDA. Such discussions would be useful for the evaluation of analytical data and may guide whether additional analytical testing would be necessary to support a demonstration of biosimilarity.

Functional assays can also provide information that complements the animal and clinical data in assessing the potential clinical effects of minor differences in structure between the proposed product and the reference product. For example, cell-based bioactivity assays may be used to detect the potential for inducing cytokine release syndrome in vivo. The available information about these assays, including sensitivity, specificity, and extent of validation, can affect the amount and the type of additional animal or clinical data that may be needed to establish biosimilarity. As is the case for the structural evaluation, sponsors should justify the selection of the representative lots, including the number of lots.

3.4.5.3 Animal data The PHSA also requires that a 351(k) application include information demonstrating biosimilarity based on data derived from animal studies (including the assessment of toxicity) unless the FDA determines that such studies are not necessary for a 351(k) application. Results from animal studies may be used to support the safety evaluation of the proposed product and more generally to support the