If the differences identified are related to the process such as the variability in the size-exclusion chromatography or the analytical centrifugation, these can be corrected with appropriate formulation changes; it is noteworthy that the Agency does not require the Q1-/Q2-type similarity of formulation common for small-molecule drugs. Realizing that the formulations of biological drugs may be under IP protection, the Agency welcomes alternate formulations as long as these do not require changing the dose or the indication. For example, an alternate route, for example, subcutaneous dosing in place of intravenous, will be a major change and does not fall within the category or similarity evaluation. The first biosimilar approved by FDA, Zarxio, has a different buffer formulation than its reference product, Neupogen.

### 4.5.3 Level 3: Highly similar

Level 3 is the minimal level that the Agency requires for a biosimilar candidate. At this point, there is high confidence in similarity in both the analytical and the functional level and further development requires securing the approval for targeted and selective clinical studies to resolve residual uncertainty. It is this residual uncertainty that needs to be understood. The uncertainty begins with first not being able to understand the source of dissimilarity. For example, if there are process-related impurities that are indigenous to any acceptable process changes, these must be fully characterized and proven to be clinically not meaningful.

In those instances where the functionality profile is not identical to in vitro bioassays or other binding assays, the Agency may require an abbreviated study to show that these differences are not impacting the efficacy or the toxicity. It is understood at this stage that any clinical studies suggested will be abbreviated, and this may not require patient participation or even efficacy trials. If suitable PD profiles are available in healthy subjects, these should be evaluated first.

In some instance, such as in the case of mAbs, the nonclinical profiling is highly species dependent, reducing the value of animal testing to reduce uncertainty. However, where robust models are available to resolve the uncertainty issues with animal studies, these should be suggested.

### 4.5.4 Level 4: Highly similar with fingerprint-like similarity

When proven to qualify for a Level 4 classification, the sponsor has already proven it to be biosimilar by the first part of the level description: “highly similar.” Now if the data presented across an orthogonally planned evaluation that all parameter studies are fully reproducible in the biosimilar candidate, more targeted studies may be required, “if residual uncertainty” remains. The last part of the definition is crucial to developing biosimilar products. What this classification means is that if