Cleaning Memo for February 2017
A Possible Approach for Biotech Limits

For years the biotech industry has argued that cleaning validation limits for biotech manufacture should not be based on the safety of the native protein, because those proteins are deactivated and degraded during a cleaning process with hot, alkaline aqueous cleaning solutions. The 2014 EMA guide on limits for "shared facilities" finally provides some regulatory support for this assertion. That guide (Section 5.3) states:

> Therapeutic macromolecules and peptides are known to degrade and denature when exposed to pH extremes and/or heat, and may become pharmacologically inactive. The cleaning of biopharmaceutical manufacturing equipment is typically performed under conditions which expose equipment surfaces to pH extremes and/or heat, which would lead to the degradation and inactivation of protein-based products. In view of this, the determination of health based exposure limits using PDE limits of the active and intact product may not be required.

This is great, but if PDE limits based on the intact active may not be required, what is required (or what is suggested or recommended)? Nothing in that section clarifies this. It has been suggested in the past (see PDA Technical Report #49) that if health-based limits could be established for the degraded fragments, that may be one way to deal more scientifically with biotech limits. The question has then been how do we determine an ADE or PDE value for the degraded protein fragments?

That said, it does appear that there may be help in Section 5.5 of that EMA guidance. That section deals with "Investigational Medicinal Products" (and not specifically biotech). It states:

> "For early development (Phase I/II) investigational medicinal products (IMPs) estimation of PDEs may be difficult based on their limited data sets. Where this is apparent, an alternative approach using categorisation into specific default value categories e.g. based on low/high expected pharmacological potency, low/high toxicity, genotoxicity/carcinogenicity, similar to the tiered Threshold of Toxicological Concern approaches proposed by Kroes et al. (2004), Munro et al. (2008), and Dolan et al. (2005)[2], can be considered to derive health-based exposure limits if adequately justified."

Note that I have left out the bibliographic references. However, one of the references (Dolan et al, 2005) is "Dolan DG, Naumann BD, Sargent EV, Maier A, Dourson M (2005). Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations. *Regul Toxicol Pharmacol*, 43, 1-9." Three of the authors were all with Merck at that time of publication. Dolan is now with Amgen; Nauamnn was one of the leaders in ISPE's Risk-MaPP. (I'm just trying to make it clear that this was a publication by recognized pharmaceutical toxicologists.)

The basic argument by Dolan et al is as follows. For "relatively unstudied compounds" with "limited or no toxicity data", an approach similar to the TTC (threshold of toxicological concern) used for genotoxic materials may be used. The tiered approach of safe daily amounts (called ADI values in the publication) is as follows.

Compounds that may be carcinogenic:	1 µg/day
Compounds that may be potent or highly toxic:	10 µg/day
Compounds likely to be none of above:	100 µg/day

What follows is my suggestion based on what is in the EMA guidance and what is proposed in the Dolan et al publication. (I want to make it clear that this suggestion is *not* put forth by the EMA or by Dolan et al, so I don't know if either would accept it.)

If limits for biotech are not set on the PDE of the active protein, then can it be set on the PDE of the degraded protein? If so, what data could be supportive? We already know that in general the immunotoxicity of proteins is lessened as the protein molecular weight decreases (see the FDA guide on "Immunotoxicology Evaluation of Investigational New Drugs" from October 2002). We can also assume that some of these degradants are present as the protein actives degrade in the human body after administration. But what other hard data is available? It would appear that degradants of biotech active proteins would fall under the Dolan et al category of compounds with limited or no toxicity data. This is a question that has to be determined by toxicologists and pharmacologists of biotech companies. If the answer is that degraded protein actives fall under that category, then the second question is which of the three tiers is appropriate? If they can come to the conclusion that the degraded proteins are not likely to be carcinogenic and not likely to be potent or highly toxic, then it seems reasonable to use the Dolan et al tiered approach and establish a PDE value of 100 µg/day. I suspect that initially this is a decision that each biotech company should make for their specific protein actives based on deactivation and degradation data for those specific protein actives.

If that value of 100 µg/day were used for carryover calculations (either for the finished drug manufacture or for equipment after the last purification step in bulk active manufacture) and then expressed as TOC, it is likely that the result would be TOC limits above typical values now used by biotech manufacturers. In other words, an appeal to the EMA guideline and the Dolan et al approach (cited in the EMA guideline), along with an assessment of where the degraded fragments fit in the tiered approach, would provide a more scientific rationale for claiming that the current TOC limits are acceptable from a patient safety perspective.

I would not recommend (at least at this time) that any firm increase their TOC limits based on this type of assessment. One of the reasons for this is that (as I have tried to argue many times) the effects of residues that we address in cleaning validation should not be based on patient safety alone; we should also consider effects on product quality (including stability, physical properties, and bioavailability of the active), which may cause the limits to be more stringent. Furthermore, for the early stages of biotech manufacture (fermentation and cell culture), I may be more concerned about effects of

residues on production efficiency and product purity due to interferences with those critical processes.

Note that there is another publication by Amgen scientists (Sharnez et al, "Biopharmaceutical Cleaning Validation: Acceptance Limits for Inactivated Product Based on Gelatin as a Reference Impurity:", *Jour Val Tech* 19:1, pp 1-8, 2013) that proposes a limit of 650 µg/dose (although this limit was not specifically called an ADE or PDE by the authors, it is possible that this could be considered as a PDE of 650 µg/*day* for degraded protein actives). That value is based on using "dosing" of a model compound, gelatin, to establish a safe dose amount for degraded protein fragments.

Now you might be thinking that there is a big difference between 100 µg/day and 650 µg/day, and see this as a problem. My response would be the Dolan et al approach is a "one size fits all" approach that is *not* limited to compounds that might be present in biotech manufacture. The Sharnez et al approach narrows the result based specifically on degraded proteins that might be present in *biotech* manufacture. It is not unlike the fact that the 0.001 dose criterion is a one size fits all approach for non-highly hazardous actives; with a PDE/ADE value for a specific non-highly hazardous active, the safe daily amount value will typically be higher than that determined by the dose criterion.

If this difference between 100 µg/day and 650 µg/day is of concern, consider calculating carryover limits with both values. If both give TOC values above what you currently utilize (which is what I expect in most cases), then stop and just use the lower value (100 µg/day).

If this approach is used, there is one significant consequence for carryover limits. That is, carryover limits will *not* depend of the dosing of the cleaned product. For example, suppose I have one biotech active that is dosed at 1 mg of active per day and a second product that is dosed at 50 mg of active per day. If the PDE value of degraded actives is 100 µg/day, that value will be used in the numerator of a carryover equation in *each* instance. While the dose of the active is irrelevant for the cleaned product, the dose of the drug product or bulk drug active of *each* product will be relevant since it is used in the denominator of the carryover equation as appropriate for the next product.

Clearly for this approach to be used, it becomes even more important to establish that the active protein is deactivated and degraded in the cleaning process. There have been numerous papers that describe lab studies that could be done to address deactivation and degradation. It may also be possible to actually measure deactivation and degradation in cleaning in commercial manufacture. Finally, additional support for deactivation can be addressed by demonstrating deactivation during any SIP process for equipment.

The purpose of this Cleaning Memo is not to say we should change our limits for biotech cleaning validation. The purpose is to help provide an even better rationale for the acceptability of current limits.