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THE NEWS THIS ISSUE

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BIOPHARMACEUTICAL IMPURITY ISSUES are drawing attention in the search for a more coherent regulatory approach to the complexities involved. With significant implications for the development and clearance of both new and follow-on biotech products, FDA and industry are evaluating their experience to date to find where meaningful impurity standards and best practices may lie. At a recent "CMC strategy forum," participants discussed the challenges of assessing and controlling the variety of process- and product-related impurities involved in developing biopharmaceuticals. ***[Included are presentations at the forum by FDA regulatory officials on: • process-related impurities (pp. 9-12) • host cell proteins (pp. 14-18) • product-related impurities (pp. 25-27), and • aggregates (pp. 28-32).]***

Process Validation vs. Specification Setting For Biologics Impurities Debated At Forum

The extent to which process validation can obviate the need for specification setting is among the key issues FDA and industry are debating in the effort to develop a more coherent regulatory approach to the complex considerations involving biopharmaceutical impurities.

At a strategy forum on handling impurities in biopharmaceutical products held in late July at the Bethesda, Maryland campus of the National Institutes of Health, regulators and industry evaluated the respective roles of process validation and specifications in controlling process-related impurities.

A consensus was reached that validating the ability of a process to remove or control impurities to appropriate levels could reduce the number of regulatory specifications needed on a biotech product, although it was recognized that the validation would not generally be substantial enough to obviate the specification need before the completion of Phase III clinical testing.

The consensus was supported by both industry and FDA representatives at the forum. In a presentation on assays for detecting biological product impurities from the host cell (*see box, pp. 14-18*), Center for Drug Evaluation and Research (CDER) Division of Therapeutic Proteins Biochemistry Lab Chief Emily Shacter expressed agency support for the process validation approach.

"You don't necessarily have to have a lot release specification for an impurity…if you can prove by other means – process validation – that you are actually removing those impurities," Shacter told forum participants. On the other hand, she noted that dropping a specification at Phase III trials is "not realistic," adding: "It is probably going to be more pre-licensure, if at all, and that still depends on your manufacturing history."

Raymond Donnelly, who works with Shacter in the Division of Therapeutic Proteins, expressed a similar point of view in his presentation on process-related impurities. "For certain impurities, testing of either the drug substance or the drug product may not be necessary and may not need to be included in the specifications if control or removal to acceptable levels is demonstrable by suitable studies," he stated.

➤ The July impurities meeting was part of an ongoing series of "CMC strategy forums" for well-characterized biological products (WCBP) being sponsored by the California Separation Science Society (CaSSS) designed to bring biotech firms together with regulators to discuss ideas and share experiences on key issues of concern.

The goal of the forums is to develop technical and regulatory consensus positions leading to better CMC-related standards and guidance in the evolving biotech arena.

The previous forum held in January focused on biopharmaceutical specification setting ("The Gold Sheet" February 2004). Two CMC strategy forums will be held in conjunction with the annual FDA/CaSSS WCBP conference in Washington, D.C. in January 2005. These will address: the analysis and structure of live virus vaccines and viral vectors; and

demonstrating comparability of well-characterized biotech products in early/late phase and post-approval.

Like the specifications forum, the impurity discussions in July concentrated on areas where the existing regulatory guidance is limited.

FDA has not issued a guidance dealing specifically with biopharmaceutical impurities. The International Conference on Harmonization (ICH) Q3 series of guidances – addressing impurities for drug substances (Q3A), drug products (Q3B) and residual solvents (Q3C) – do not apply to proteins or other biological products. ICH's Q6B on specification setting for biotechnology products does address impurities briefly, but the material is relatively basic. Some discussion relative to impurities is also provided in the ICH Q5 series on biotech products, including Q5C on stability testing and Q5D on cell substrates.

- The resolution of impurity concerns are critical to developing and getting clearance for new biopharmaceuticals as well as to creating a foundation for follow-on biopharmaceuticals.

On September 14-15 in Rockville, Maryland, FDA held the first of a series of public workshops to help plan for the development and regulation of the follow-on products. The workshop was focused on gathering industry input on scientific and technical issues related to manufacture, characterization and immunogenicity.

FDA was close to issuing a draft guidance on the application requirements for follow-on biopharmaceuticals last spring ("The Gold Sheet" January 2004), but has delayed doing so to accommodate further exploration of the issues at the workshops. FDA is also delaying regulatory decisions on applications. In mid-September, the agency announced that, although it had completed the review of Sandoz' marketing application for ***Omnitrope*** (a follow-on version of Pfizer's human growth hormone biologic ***Genotropin***) it was delaying a final approval decision until later next year while the agency further considers the legal and scientific issues involved in assessing protein comparability.

During the first day of the July forum, participants discussed strategies for evaluating and controlling process-related impurities, focusing specifically on host cell proteins during the afternoon session. Product-related impurity issues were addressed on the following day, with specific attention given to protein aggregates during the afternoon.

Specification Setting Seen As Phase-Dependent

In line with the discussions at the January forum, the issue of when and how to set specifications received considerable attention at the July impurities sessions.

The specification issue is an important one to biotech manufacturers who are looking for ways to maximize the effectiveness of their quality control resources in the face of the heavy demands of producing and evaluating the complex products. Reducing the on-going batch testing burden and potential regulatory encumbrances that specifications entail is viewed as an important component in optimizing resource usage and avoiding unnecessary production delays.

[EDITORS' NOTE: The dialogue on biopharmaceutical specifications will continue at an FDA/ industry workshop in Washington, D.C., Oct. 6-8 (*www.aapspharmaceutica.com/specifications*).]

The potential for process validation to delimit specification requirements was seen by participants at the July forum as hinging on how much is known about the level of a given impurity during the development and clinical testing process and its linkages with the clinical consequences.

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F-D-C REPORTS, INC.
5550 FRIENDSHIP BLVD., SUITE ONE
CHEVY CHASE, MD 20815-7278
PHONE 1-800-332-2181 FAX 301/664-7258

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Genentech Division of Recovery Sciences Director Gregory Blank summarized the dominant thinking among meeting attendees in saying that understanding of the impurity during a process is phase-dependent. "Inevitably you are not going to have the breadth of information on your process in your initial clinical trial stages that you would at the time of license application."

As such, many manufacturers opt to conduct end-product testing to assess the full range of possible impurities in the earlier development phases, but then later remove some testing through process validation.

Forum participants agreed that tests probably would not be removed until the stage of submitting a marketing application after completion of qualification lots. Blank pointed out that, while there may be some variation among drug development processes of different companies, "as a general rule, not everyone has all of their process validation studies…complete to the extent that they decide whether to drop [an impurity test] at the start of Phase III trials."

➢ CDER's Donnelly commented that it has been FDA's experience that specifications, including those for process-related impurities, are often set broadly in the earlier stages of product development, and that with time, "as manufacturing experience is gained, we encourage manufacturers to strive to narrow the specifications for their products."

"The manufacturing process should be well-defined before a sponsor begins their Phase III clinical study," Donnelly advised. Further, processes "should be evaluated to identify potential sources of process-related impurities as early as possible so if a biologic is moving forward in the clinic and a sponsor is planning to submit a BLA [biological license application], that they already have a good handle on the types of process-related impurities that may be characteristically associated with their manufacturing process."

FDA recognizes that "some manufacturing processes can achieve lower levels of process-related impurities than others," Donnelly acknowledged, but the agency "expects all manufacturers to strive to continually minimize the levels of process-related impurities in their products."

Decision Tree For Acceptance Criteria Proposed

Donnelly's presentation at the Monday morning session of the forum included an overview of the different types of process-related impurities typically seen by biotech manufacturers – including those derived from the cell substrate, the cell culture, as well as from downstream processing – and the analytical techniques used to measure them (*see box pp. 9-12*).

The CDER official stressed that determining the purity of a biologic drug substance is highly method-dependent, and that manufacturers usually employ "two or three distinct analytical methods to qualify the purity of their product."

Donnelly was joined in the opening session of the forum by Amgen Analytical Resources Laboratory Head Heather Simmerman, who outlined a decision tree for setting acceptance criteria for process-related impurities, and Genentech's Blank, who talked about his company's strategy for process impurity removal.

➢ Based on suggestions made in the ICH Q6B guidance, Simmerman's decision tree is intended to help biologics manufacturers develop a strategy for deciding when and how to set an acceptance criterion or limit for a process-related impurity (*see box, p. 4*).

In the discussion following her presentation, Simmerman clarified that the steps in the decision tree do not necessarily need to be followed sequentially but are considerations that manufacturers may address "concurrently."

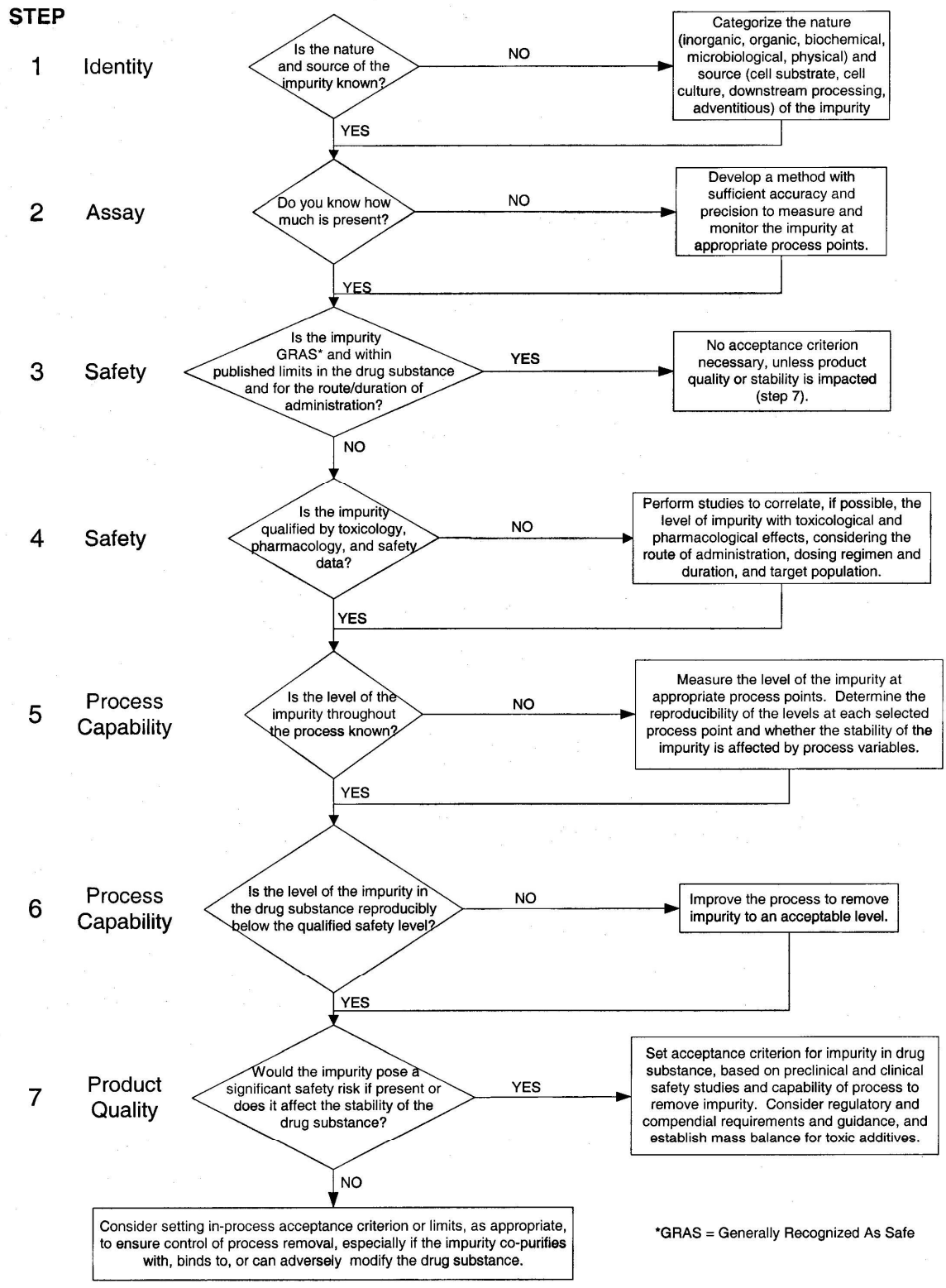
The structure and content of the decision tree, as well as its usefulness as a tool for manufacturers in obtaining acceptance criteria for impurity levels, garnered support from the meeting participants. At the end of discussion for that session, most attendees responded affirmatively when asked whether the decision tree captured the correct questions and whether it could be useful.

➢ Blank addressed the role process characterization can play in understanding process capability and in helping manufacturers maintain reproducibly low impurity levels.

He emphasized the large degree of heterogeneity in the types of process impurities observed. While it is sometimes tempting to group process impurities together, Blank said, "they are extremely heterogeneous and present a variety of challenges from the process design perspective." He added that "multiple [process] steps with orthogonal mechanisms" are generally required to get the impurities down to acceptable levels.

Decision Tree For Determining Process-Related Impurity

At the impurities strategy forum in July, Amgen's Heather Simmerman presented the following decision tree, intended to help biologics manufacturers with when and how to set acceptance criteria for process-related impurities.



Blank advocated the potential benefits of "pilot plant consistency runs" in process characterization. The pilot plant process employs the same parameters as the commercial manufacturing process and takes place perhaps during Phase III trials, he explained.

"This will help you determine the natural variability of the process performance," Blank said, "and it adds to your database at the time you submit your BLA [biological license application] – you have got some commercial scale runs, but you can also supplement that database with regard to process performance with these pilot plant runs."

➢ Another process characterization strategy is to intentionally vary process parameters and assess their impact on product quality and process performance, Blank explained.

Typically run at a laboratory scale, this approach involves taking the various parameters associated with a particular manufacturing unit operation and varying them to extremes "far beyond where you would normally want to operate to understand what the process robustness is," and what is the effect on process-related impurities. The parameters could be varied either singly or in combination, he added.

"Extensive characterization studies can demonstrate what the process capability is for removal of process-related impurities over a wide range of process parameters," Blank said, and likely will help manufacturers better understand whether impurity removal can be done through process validation, in-process monitoring, or whether "you want to actually have this as a bulk specification."

[EDITORS' NOTE: Blank discussed Genentech's "integrated approach to bioprocess validation" at the CaSSS/FDA WCBP symposium in January 2004. An excerpt of that presentation is included in the January 2004 issue of "The Gold Sheet."]

Impurity Target Levels Relation To Safety Debated

During the discussion period on process-related impurities, participants investigated the implications of product safety on setting target levels for impurities, and whether general guidelines on targets are possible despite differences in biotech products and processes.

They also traded ideas about collecting data from across companies about impurity levels and the possibility of establishing industry-wide standards for methods and specifications.

In addition to whether validation or monitoring could replace bulk specifications, the discussion was guided four other questions: ● Which process impurities or classes of impurities need a specification? ● What factors determine the acceptance criteria for clearance? ● Is there a target level for clearance and is the target dependant on phase of development? and ● At what point in the process should clearance occur?

Surveying the audience, Simmerman found that few of the participants' companies kept "predetermined lists of impurities that would always get a specification" or worked with impurities that received specifications "no questions asked." Most participants also did not have fixed target levels for clearance, but adjusted the targets as a function of the stage of development.

➢ Participants agreed that the four factors listed in the ICH Q6B guidance and included in Simmerman's decision tree – assay capability, process capability, safety and product quality – were generally used to determine the acceptance criteria for impurities.

While firms do not have set targets already in mind, they do create lists of potential impurities "at the very beginning when they know they have a product candidate," Simmerman summarized. Before going into Phase I development, "they are going to map out their process and have a pretty good idea of what they are going to need to look for."

MedImmune Analytical Biochemistry Senior Director Mark Schenerman maintained that most companies evaluate their methods "along with their process in parallel." The initial evaluation is important because it allows firms to "get the order of magnitude of where you want to be with regard to sensitivity for your assay, and that gives you a starting place for selecting which methods are going to be appropriate."

Schenerman also stressed the importance of FDA involvement during process development: "It is a continuous dialogue between the sponsor and the FDA as you move through clinical trials, because your initial assessment of course is going to be important, but the feedback from FDA is going to be important also."

In-House vs. Commercial Assay Methods

The participants at the forum were also surveyed by Simmerman on the use of in-house vs. commercially available impurity assay methods.

There was a "strong consensus" that manufacturers generally start developing proprietary methods in house at the IND stage. Use of commercially available methods and kits was also reported to be common. About half of the audience indicated that their companies incorporate a "generic" assay strategy for those that can be used across processes/products.

John Ivancic from Eli Lilly noted that at the IND stage, Lilly generally uses a combination of commercial kits and proprietary methods, "depending on what we are trying to measure." A commercial kit, for example, might be employed for "something like a host cell protein or things like transferrin," once it has been qualified to "make sure that it measures a reasonable level."

➢ "What we have seen at the FDA," Donnelly commented, "is that sponsors/manufacturers will each develop their own antibodies for detection of host cell proteins, and that creates the potential for a lot of variability from manufacturer to manufacturer. One manufacturer's assay might be much more sensitive than another."

To help address the problem, Donnelly asked participants if interest existed in an "accepted central assay" for a given variant, adding that it might be cost-effective for a small biotech firm "to be able to utilize or to access a centralized laboratory that is qualified to conduct such assays."

The centralized lab concept was greeted cautiously by one respondent: "Having, for example, a host cell protein assay in which you had to use a centralized laboratory – send materials out and wait for data to come back – the turnaround time might be limiting." On the other hand, he said, "if there was a standard that would be made available, that I think would be very useful."

➢ Donnelly noted that there are currently no internationally recognized standards for process-related impurities with the exception of DNA, for which there is a World Health Organization (WHO) specification.

Amgen representative Joseph Phillips asserted that there is a "driving force" for establishing international assay standards. "It would be good to actually help us in terms of a single methodology's lot disposition," he said, "so that we are not cherry-picking or having two sets of specifications based upon the European needs versus the U.S. needs."

Phillips discussed Amgen's experience with standardizing assays on Chinese hamster ovary (CHO) proteins and DNA, noting that FDA may offer more flexibility than its European counterpart.

"We can, in fact, discuss with the FDA the ability to specify these impurities at the impurity profile stage. Throughout the process we would have an in-process control and we can actually validate them out. I think...the FDA is really, really willing to discuss that with us – to say that maybe by the time you get your fourth column of your purification you are fine to demonstrate that you have controlled this to the level that you want. But for the European filings we are finding that they want to have a specification for the actual filtered purified bulk. That then has to go onto the release specs."

Phillips characterized the assay methodology standards issue as a "chicken and egg situation: You want to have an international spec which is common, but then we are looking for differences with the methodologies predicated by the heterogeneity of the materials and the different approaches to the methods." He suggested that the situation could be aided by forming a consortium involving companies with a vested interest in the end point.

MedImmune Vaccines' Hersh Mehta commented that he agreed "conceptually" with the development of common assay standards, but cautioned that "technically, it is a challenging problem." For example, he noted that any consortium would need to reconcile possible differences in the origins or gene expressions of each company's *E. coli* or CHO cells. "Host cell proteins from one company's conditions to other companies' conditions could vary considerably," Mehta added, asking: "How would one standardize and apply this?"

➢ Phillips provided an example to demonstrate that an internationally recognized set of standards is both needed and possible.

When Immunex and Amgen joined about two years ago, "they had completely different approaches towards, say, Protein A. We have managed to come to

a common set of standards/reagents, with just those two organizations...So I don't think it is not achievable. It is just a case of: (a) first of all deciding that this is a good goal, and then (b) wanting to contribute to the desire and the effort to accomplish that."

➢ Simmerman suggested that the utility of assay standards may be phase-dependent.

"I can see that it would be very easy to implement these quite quickly" early in development, but "depending on the circumstance" and issues identified during the process development, a standard "may not suffice later on," she said. The question is, "is the utility and the effort that we would put in establishing it warranted for that early development?" Simmerman "suspect[s] so, because candidates will drop out and that is really a very costly part as well of our development."

Standardization efforts are also taking place elsewhere in the world. Dougherty commented that an organization from the United Kingdom called the Pharmaceutical Analytical Sciences Group (PASG) is "actively in discussion about establishing reference material for host protein cells." He mentioned that a U.K.-based Lilly colleague informed him before the forum that the PASG viewed the forum discussions as an opportunity to build "consistent dialogue across the pond" on international standards. The PASG will be meeting in the U.K. on October 11-12 (www.pasg.org.uk).

Acceptable Impurity Levels At Issue

Participants generally supported the concept of setting industry-wide target levels for common process-related impurities, where manufacturers' process validation exercises would have to demonstrate clearance of the given impurity to below that target.

FDA representatives advocated for a move toward internationally recognized specifications for certain common impurities. The value of doing so is seen as bringing uniformity and consistency to the way manufacturers handle impurity specs.

Several suggestions were made about previous work that could serve as models for moving toward universal standards for impurity levels. A parallel was drawn to an International Conference on Harmonization-led effort to categorize residual solvent impurities for drug products, where databases of toxicological studies were examined with the hope of linking exposure limits to dosage levels. A similar path could be taken for well-known process-related impurities that appear in many biopharmaceutical manufacturing processes across companies, meeting attendees asserted.

It was suggested that a model that could be considered is the WHO-recommended specification for levels of nucleic acids in biological products. Other participants suggested additional sources of information for use in establishing target levels, including references for clinical levels of substances classified as "generally regarded as safe (GRAS)," and existing data for excipient compounds in drug and biotech products. Guidance from regulatory agencies on impurity levels has not been issued.

➢ Determining what are acceptable levels for process-related impurities became a central concern during the first discussion session.

Amgen's Simmerman was among those who noted the importance of the dose level, dosing schedule, route of administration and patient population in considering appropriate impurity levels. She related that manufacturers are concerned with setting standardized target levels because different target levels could be appropriate for different patient populations, for example. While a higher level might be acceptable in some cases, once a lower level is set, manufacturers would be required to meet that possibly more stringent standard.

The question, she said, is "how well do we need to correlate the level of the impurity with [associated] toxicological or pharmacological effects in consideration of the route of administration, dosing, regimen duration and the target population?"

CDER's Shacter emphasized the large impact that both dosing and administration route have on making determinations of acceptable impurity levels and cautioned that these factors will preclude a "one-size-fits-all" approach to setting general specifications.

FDA's Kathleen Clouse, acting deputy director of CDER's Office of Biotechnology Products, commented that while an impurity level could be acceptable for a given target population, the possibility that the product would be used by other patient groups in the future should be considered.

"A general recommendation," she stated, would be to make impurity level "as low as reasonably possible, so that it is not just linked to one particular patient population." Another meeting participant expressed agreement, adding that a product aimed initially at a "normal"

patient population could find applications later in pediatric or geriatric patient groups, who might exhibit heightened sensitivity to certain process-related impurities, and therefore would require lower levels for safety.

Simmerman raised the further issue of how much safety data is enough to be sure that a given impurity level is acceptable. She pointed out that knowing when a manufacturer has enough data to ensure an appropriate specification is very difficult with protein products. "How many patients do you need in the early stages to understand that?" Simmerman asked.

Discussion of the magnitude and diversity of the population that is sufficient to understand the safety of a biological product turned back to the concept of sharing information on impurity levels and safety data between companies and the mechanism through which to do so. A consortium-type structure was proposed as a means for companies to share information in a collective database.

Simmerman suggested that an organization like the Product Quality Research Institute (PRQI) serve as the vehicle for collecting and disseminating shared data. Involving an impartial third-party interest would allow a greater degree of confidentiality for those companies who participate and could possibly alleviate the concern over revealing proprietary information held by many firms.

CDER Office of Biotechnology Products Acting Director Keith Webber questioned how much the agency could use information on the effects of a contaminant from one company when evaluating similar products from other companies.

Among the concerns raised on measuring and setting acceptable levels for GRAS substances was the point that manufacturers and regulators still must consider the substance's potential impact on the product. For example, a safe metal ion for human consumption could potentially activate a protease enzyme that in turn degrades product.

➢ Product safety as it relates to the need for specifications is addressed in the draft decision tree presented by Amgen's Simmerman.

The last step of the decision tree, dealing with overall product quality, asks whether the impurity would pose a "significant safety risk" or would affect the stability of the drug substance. If the answer is yes, Simmerman commented, "I think clearly an expectation would be for a specification."

For those impurities posing safety risks, the decision tree suggests setting an acceptance criterion "based on preclinical and clinical safety studies and capability of the process to remove the impurity." Further, the protocol says regulatory and compendial requirements, as well as available guidance should be considered in setting specifications. A final suggestion is to establish mass balance for toxic additives.

If the impurity does not pose a significant safety risk, the decision tree says manufacturers should "consider setting in-process acceptance criteria or limits, as appropriate, to ensure control of process removal, especially if the impurity co-purifies with, binds to, or can adversely modify the drug substance."

Should Targets Reflect Safety Or Process Capability?

Forum participants took up the question of whether purification of products should be driven only by safety, or if process capability should govern how much impurity should be removed after safety concerns have been addressed.

Pointing out the sometimes arbitrary nature of the impurity target levels, then CDER Office of Biotechnology Products official Anthony Mire-Sluis said "I think we are going more down the route of 'well, company A can get their Protein A down to a certain level, then it becomes an industry expectation.'" Mire-Sluis left FDA to join Amgen in August as head of product quality and external affairs.

"That's the balance I would like the audience to think about – is it the safety really, or are we just ending up with numbers because you *can* get rid" of protein impurities, Mire-Sluis said. He questioned whether FDA should have the authority to require that a company remove an impurity down to a certain level if patient safety is not affected. "Are we now tying ourselves as an industry to process capability and it is just lowering the levels to meaninglessness, or is it truly linked to patient safety?" he asked.

Amgen's Heather Simmerman pointed out that there is a cost effectiveness component to driving impurity levels lower (potentially unnecessarily) and to measuring them.

Simmerman also cautioned that the potential impact on product supply should be considered as a company drives toward lower and lower impurity levels. Setting the target too low may unnecessarily risk batch rejection and jeopardize the supply for patients who need it.

On the other hand, it was also noted that, in general, fewer impurities are correlated with higher safety. Shacter pointed out that another major advantage to having a cleaner product overall, even when safety concerns may not be an issue, is that the ability to make a change in the manufacturing process and then to evaluate that change is made easier by lower impurity levels.

Another participant called for further regulatory agency guidance on what are appropriate levels of impurities for particular products based on the experience observed by the agencies.

CDER's Donnelly On Process-Related Impurities

At the July strategy forum, Raymond Donnelly of CDER's Office of Biotechnology Products gave the following presentation on "Evaluating the Potential Impact of Process-Related Impurities on Product Quality." Donnelly addressed definitions, types of process-related impurities, and purification and evaluation, and gave some examples of common impurities and the associated methods.

What I would like to do during the time that has been allotted to me this morning is to provide sort of a brief overview of some of the types of process-related impurities that we often encounter in terms of our review processes at the FDA, and hopefully use this as a forum to stimulate an exchange of discussion by manufacturers such as yourself with those of us on the panel.

DEFINITIONS

Let me start with a few definitions. Most of this information has been culled from the ICH guidance documents and is probably intimately familiar to most of you.

The **relative purity** of a biological product is normally expressed in terms of its specific activity; that is, units of biological activity per milligram of purified product. However, the determination of purity of a drug substance is highly method-dependent, and typically we see that manufacturers will choose two or three distinct analytical methods to qualify the purity of their product.

A word about **product variants**: The purified drug substance itself can be composed of several distinct molecular entities, or 'variants,' so that a purified drug substance does not necessarily imply, or mean, a homogeneous single population of a single protein. When these variants are derived from anticipated post-translational modifications, they may be considered part of the desired product. When they are formed during the manufacturing process or upon storage, they are also considered product-related substances.

Impurities: In addition to evaluating the purity of the drug substance and drug product, manufacturers should also strive to identify and quantify impurities. These impurities may be either process-related or product-related.

Process-related impurities include those that are derived from the manufacturing process itself, and these can include, for example, cell substrate-derived impurities – host cell proteins, host cell nucleic acids, which I will not discuss much at all during the morning session as it will be discussed this afternoon….Process-related impurities would also include cell culture components. These would include agents such as antibiotics, chemical induction agents such as IPTG or agents such as methotrexate which are used to maintain plasmid expression, or certain medium components – transferrin, insulin, etc. And finally, downstream process-related impurities would include things such as organic solvents and column leachables, and I will talk a little bit more about some of these shortly.

Product-related impurities include molecular variants that arise during manufacturing and/or storage that do not have properties comparable to those of the desired product. So these would include product precursors, degradation products such as dimers, trimers, oligomers, and various oxidized forms.

And finally, **contaminants** are defined as adventitiously introduced materials that are not part of the manufacturing process itself. These include agents such as mycoplasma, bacteria, and viruses. All manufacturing processes should strive to include appropriate steps to prevent, remove, or inactivate such contaminants. And obviously one of the major concerns for any manufacturing process is control of endotoxin. The removal of endotoxin is normally demonstrated through process validation and is typically accomplished through a variety of chromatographic steps during product purification. So even for a recombinant protein that is expressed in E. coli in which there is a very high level of endotoxin at the initial cell lysis stage, through process validation and a well defined purification process manufacturers have very successfully been able to demonstrate substantial removal of endotoxin.

And finally, let me just say a word about **specifications** because it relates to what Heather [Simmerman] had mentioned previously in terms of when is it appropriate for a manufacturer to establish a specification for a process-related impurity.

So much of how we define specifications for qualification of drug substance and drug product also apply to the establishment of specifications for process or product-related impurities. So, again, as defined by ICH guidance documents, specifications are a list of tests, analytical procedures, and appropriate acceptance criteria which specify the numerical limits, ranges, or other criteria for such tests. These specifications establish a set of criteria to which a drug substance, drug product, or intermediate at intermediate stages of manufacturing should conform to be acceptable for its intended clinical use.

Acceptance criteria are established based on data obtained from lots used in preclinical and/or clinical studies. Whenever possible the product should be compared with an appropriate reference standard. And it has been our experience with regards to the biologics at the FDA that typically a manufacturer at the early stages of product development in Phase I, Phase II, and preclinical stages, that specifications are often set fairly broadly and that, with time, as manufacturing experience is gained, that we encourage manufacturers to strive to narrow the specifications for their products.

The same would hold true for a specification for a process-related impurity. Acceptance criteria for process-related impurities should be established based on data obtained from lots used in the preclinical and clinical stages of product development. So again, as manufacturing experience is gained, we would expect that specifications for process-related impurities or for product-related impurities would be tightened. When adequate quantities of an impurity are present, the manufacturer should strive to characterize and set acceptance criteria for such impurities.

TYPES OF PROCESS-RELATED IMPURITIES

So why measure process-related impurities? Evaluation of process-related impurities is part of the well-defined manufacturing process and provides assurance to the FDA that such impurities do not compromise the quality or safety of the final product.

And I have already mentioned, let me reiterate, there are several types of process-related impurities. These would include cell substrate-derived, cell culture-derived impurities, and downstream processing-related impurities. So it is sort of paradoxical that a purification process in itself can introduce impurities.

Cell substrate-derived impurities include but are not limited to host cell protein or nucleic acids, principally DNA, derived from the host organism. For host cell proteins, typically a sensitive immunoassay is normally used to detect a wide range of protein impurities, and I am sure that this issue will be discussed in some detail later this afternoon. For host cell nucleic acids, typically hybridization techniques are normally used.

Cell culture-derived impurities include, again, things such as: antibiotics; gene expression induction agents such as IPTG; fetal bovine serum used in the cell culture phase is a significant concern in light of particularly heightened concerns in recent years with regards to potential BSE [bovine spongiform encephalopathy] contamination in agents that are used in cell culture processes – in particular, those derived from bovine sources; and finally other medium components such as insulin and growth factors that may either bind to the drug substance or impact on its quality in some way.

Downstream processing related impurities might include things such as: enzymes – for example, an enzyme that is use to proteolytically cleave a precursor to generate a bioactive form of the protein; chemical agents such as cyanogen bromide, guanidine HCl; oxidizing and reducing agents; inorganic salts such as heavy metals and arsenic; organic solvents; ligands used to capture specific monoclonal antibodies [and] conversely monoclonal antibodies that are used as affinity substrates for binding specific ligands; and finally, column leachables, in particular protein A, which I'll say a little bit more about in a moment.

PURIFICATION AND EVALUATION

OK, to paraphrase from the **ICH** S6 document: 'There are potential risks associated with host cell contaminants derived from bacteria, yeast, insects, plants, and mammalian cells. The presence of cellular host contaminants can result in allergic reactions and other immunopathological effects. The adverse effects associated with nucleic acid contaminants are theoretical but include potential integration into the host genome.'

And finally [from ICH Q6b]: 'For certain impurities, testing of either the drug substance or the drug product may not be necessary and may not need to be included in the specifications if efficient control or removal to acceptable levels is demonstrable by suitable studies.' So in other words, if a manufacturer can demonstrate through process validation that they have reduced the levels of a process-related impurity to an extremely low level, and can consistently demonstrate from lot to lot that their process achieves that reduction, it may not be necessary to set either an in-process specification or a final specification for clearance of the process-related impurities.

This is a hypothetical example, this is not taken from anyone's IND or license, this is just to show you the sorts of **purification schemes** that we typically see and review. In this case a frozen cell phase, perhaps from an *E. coli* homogenate, is lysed. There may be a homogenization step followed by centrifugation, solubilization and centrifugation, a reduction and acidification step, a refold step, then several column chromatography steps. Typically we see two or three ion exchange chromatography steps in a typical purification process for a recombinant therapeutic protein. And finally the purified drug substance would be formulated and ready for preparation of the final drug product.

You can see that a scheme like this provides the potential for introduction of a number of process-related impurities. So the question is how does a sponsor assay for process-related impurities? And many of you are experts in this, but let me just, to stimulate some discussion, mention a few examples.

In the case of **media components** used in cell culture – again, these would include agents such as transferrin, insulin, and albumin – these are typically measured by a sensitive amino assay – a radioimmunoassay and ELISA, or sometimes Western blotting. Chemical additives such as antibiotics, methotrexate, or guanidine HCl would be measured by ELISA, immunoassays or more typically by HPLC [high-performance liquid chromatography] methods. And column leachables such as protein A or heavy metals that may leach: in the case of protein A it is typically measured by ELISA; heavy metals by atomic absorption.

Chemical impurities are added to control cell growth during cell culture, to amplify gene expression, or to prevent the growth of microbial contaminants. So there are a number of applications. Because low molecular weight chemicals are relatively non-immunogenic, these impurities are usually measured by HPLC methods, because it is often difficult to generate an antiserum or monoclonal antibody specific for that product – not impossible, but there are established biochemical methods such as HPLC that are more suitable for that application.

EXAMPLES

Let me now just mention a couple of common examples of process-related impurities and some of the methods that are used to measure those.

The first is **guanidine HCl**, which is a well-known denaturing agent. As a low level process-related impurity, guanidine HCl can cause dermal irritation. At high concentrations it can cause neurological effects. So obviously control of guanidine HCl levels and demonstration of its removal, or substantial reduction, is a significant concern. To monitor guanidine HCl in process intermediates, manufacturers normally use strong cation-exchange columns coupled with a conductivity meter. And this is an example. This shows you an experiment in which in this case they could detect guanidine HCl at 25 nanograms per ml using a very sensitive HPLC method.

The next example I wanted to mention is **methotrexate**. This is commonly used as a cell culture medium additive to select for transformed cells that contain a transfected gene. Methotrexate is a folic acid antagonist. There are well known renal toxicities and bone marrow toxicities that are associated with methotrexate when it is administered actually for certain clinical indications. As you may know methotrexate is, for example, used in treatment of rheumatoid arthritis, so there is a significant reference base for understanding the biological effects of this compound. Several methods are typically used to measure methotrexate. These would include HPLC, fluorescence polarization and enzyme inhibition assays.

As I mentioned, **column leachables**: The means by which a product is purified can introduce process-related impurities. Column resin decomposition, for example hydroxyapatite chromatography, can result in leaching of calcium phosphate crystals. Fortunately these can usually be removed through one or more additional downstream processing steps.

In preparing this talk I had some discussions with my colleagues in the division of monoclonal antibody products, and I got a little background information on **Protein A**, which is commonly used as an affinity matrix for capturing monoclonal antibodies. Protein A as you probably know is a 42 kilodalton protein isolated from *Staphlyococcus aureus*. It specifically binds both human and mouse antibodies. Consequently it is commonly used to purify immunoglobulins from various sources. As an impurity, Protein A can potentially stimulate immunologic, mitogenic, or anaphylactic responses in humans. So again, monitoring processes for the levels of Protein A is very important.

Protein A is a process-related impurity associated with the manufacturing of many monoclonal antibody products. Normally this is measured by immunoassay. In discussions with Kurt Brorsin and Patrick Swann in [the Division of Monoclonal Antibodies (DMA)] it is my understanding for the products that are regulated in DMA that manufacturers can either validate its removal through the manufacturing process, or quantitate the levels of Protein A as a release specification. And typically sponsors are encouraged to strive to achieve levels on the order to 10 to 12 parts per million.

Finally, **bovine IgG** is a process-related impurity that is often difficult to separate from the desired monoclonal antibody product. Manufacturers can reduce the levels of this impurity by minimizing its use during cell culture; that is, DMA typically recommends that sponsors strive to use serum free-culture conditions for manufacturing monoclonal antibody products. And another approach is to maximize the loading of human IgG or an IgG fusion protein on an affinity column to minimize the binding of any bovine IgG that might co-purify with the desired monoclonal antibody product.

So in **conclusion**, the manufacturing process should be well defined before a sponsor begins their Phase III clinical study. Manufacturing processes should be evaluated to identify potential sources of process-related impurities as early as possible, so that if a biologic is moving forward in the clinic and a sponsor is planning to submit a BLA application, that they would already have a good handle on the types of process-related impurities that may be characteristically associated with their manufacturing process. And appropriate limits for process-related impurities should be established when appropriate.

We recognize that some manufacturing processes can achieve lower levels of process-related impurities than others. However, the FDA expects all manufacturers to strive to continually minimize the levels of process-related impurities in their products.

Let me just acknowledge several of my colleagues in the Division of Monoclonal Antibody Products and Division of Therapeutic Proteins who either contributed some additional information or slides to my presentation These are Kurt Brorsin and Patrick Swann at DMA and Emily Shacter and Barry Cherney at the Division of Therapeutic Proteins.

Generic HCP Assays Usable If Validated

Debate at the second session of the forum on host-cell proteins (HCPs) centered around the relative advantages and disadvantages of commercial HCP assays compared to "in-house" assays developed by a firm to investigate HCPs either in one specific product, or in a set of multiple products.

A consensus emerged that generic assays for host cell proteins are usable provided they have been sufficiently validated. Meeting participants agreed that the reagents used in a generic-type assay should be qualified along with the assay itself to ensure that the antibodies are in fact recognizing host cell proteins.

➤ Joining CDER's Shacter in giving presentations at the HCP session were Genentech analytical chemist Kathleen Champion and Biogen Idec's Helena Madden, who shared approaches of their respective companies to handling host-cell proteins.

Champion explained Genentech's development of assays capable of measuring host cell proteins from *E. coli* as well as Chinese hamster ovary host cells. She said the company's method of monitoring residual host cell protein levels with ELISAs provides a "good means of measuring manufacturing consistency to make sure we are in the right ballpark," but the ELISAs should be used in a complementary way with other gel assays and Western blots to generate the full range of available information.

Madden took forum participants through the advantages and disadvantages of generic HCP assays as opposed to process-specific assays using a case study from her company involving polyclonal antibodies.

She affirmed that biologics manufacturers could use commercial kits for measuring host cell protein impurities "in principle," but they "have to show that the assay is doing what you think it is doing," including validation of its suitability for the particular production strain and its sensitivity.

➤ However, Shacter cautioned that CDER's experience to date with commercial assays "is not ideal" because of lower sensitivity and less compatibility with the cells involved in a particular process.

Champion corroborated Shacter's assessment in the discussion that followed. She said that comparisons done by Genentech between commercially available kits and the company's own in-house generic assays "found the kits were much less sensitive."

Shacter summarized the tenor of the participant comments: "What I'm hearing is that the commercial kits are not yet ready for prime time." The CDER official stressed that "that is a practical message – it is not something that FDA wants to impose on anybody." She further stated that although industry experience with commercial kits shows limitations in their effectiveness at present, this situation could change over time and "FDA will certainly be happy to change our perspective."

➤ In addition to addressing issues involving HCP assays and validation, the forum participants also tackled the question of what level of HCP impurities is "acceptable."

Champion reiterated a number of factors that need to be considered when deciding on acceptable levels for HCPs, including the fact that different assays have different degrees of sensitivity. In addition, as was pointed out for other process-related impurities, the dose level and frequency as well as route of administration must be considered.

In discussing the issue of setting "acceptable" impurity target levels, Genentech's Blank noted that host cell proteins present complications in that an HCP is not a "discrete chemical entity" like some other common impurities. He added that cross-company comparisons of HCP ELISAs would likewise be difficult because of the multitude of variables associated with quantifying HCPs.

Shacter commented that FDA would "look favorably" upon manufacturers who use both ELISAs and Western blots at least in the validation of the HCP assay, citing the value of the former to qualify the latter.

CDER's Shacter On Evaluating Host Cell Proteins

In a presentation at the July strategy forum on evaluating host cell proteins, CDER Division of Therapeutic Proteins Biochemistry Lab Chief Emily Shacter addressed: • the purpose of HCP assays • the types of assays being used • setting HCP specifications and • additional points of regulatory concern.

PURPOSE OF HCP ASSAYS

What I will do is give you a brief and general overview of some of the FDA perspectives. First…why do we have host cell protein assays at all? The points that I can think of, and there might be some others that I have left out are: • to control product purity • to look at your consistency of manufacture as part of your process validation, and, • to be able to understand immunogenicity issues that might come up during the clinical trials. I will delve into these a little bit more deeply.

First, for **assessing product purity**: The great advantage of using a specific host cell protein assay is that you can detect lower HCP levels using one of these assays than you can for your standard protein characterization technique. So for example if you are using reverse phase HPLC or size exclusion HPLC, generally the limits of sensitivity are about 0.5% to 1%. If you have, for example, a ppm or 100 ppm of host cell proteins in your product, then you really can't see that. So if you think of a silver stained gel, for example, where maybe you are loading a microgram of total protein, and you only have a nanogram of sensitivity – a nanogram divided by a microgram is a part per thousand. So the general techniques for looking at protein impurities really aren't strong enough, and we need something that is really zooming in on the host cell proteins.

Also, just to define the term ppm: I know that sponsors may have some different ways that they use it. I will be using it as being parts per million where it is a quantity of protein per quantity of the product protein. So for example, as I had just said, if you have a nanogram of host cell contaminant and you have a milligram of your protein product then that would be a ppm, OK? But I know that sometimes sponsors use it as per milliliter of water, i.e. micrograms per milliliter of water – since a milliliter of water weighs a gram. It is probably best to have it be per protein.

To say the obvious, host cell protein assays are cell strain specific. So if you are using *E. coli*, CHO cells, yeast, insect cells, or some human cells, then your host cell protein assay will be targeted at that species for the source manufacturing.

One of the great benefits of a host cell protein assay then is to help you distinguish between a host cell contaminant and a product-related impurity. For example, if you are looking at a general impurity profile and you have maybe a couple of bands on your Western blot, (that is your anti-product [antibody] Western), and you see some bands that you are not sure if they are actually product-related or if they might have come from the host cell, then you can have your host cell protein assay doing a Western blot and overlap those two. The antibody that you use for detecting your product will only be as clean as your product. So if you have host cell proteins in the immunogen that goes into making the antibody that you are using for your anti-product Westerns, then you may see host cell proteins in there as well. If you have a bona fide antibody that is just to the host cell proteins (like from a null cell source), then you can tell which of those bands might be host cells versus which are product.

The second main reason, and a very important reason, is **consistency of manufacture** and is part of your process validation. As we discussed already earlier this morning, sometimes you might have an HCP specification for lot release, but as Q6B says, and Ray [Donnelly] showed this in a slide earlier, the bottom line of the paragraph is that you don't necessarily have to have a lot release specification for an impurity…if you can prove by other means – process validation – that you are actually removing those impurities. So then you might not have to have a specification. This is probably not possible in early phases of product development when you don't have any manufacturing history and you are just working out your assays. But in order for this to be true you do have to have a validated assay to be able to say, 'yes, we have removed 99.9% of the host cell proteins.' Well that, again, is based on the quality of your assay. So your assay has to be doing what you think it is doing in order for us to know there aren't any host cell proteins or that you know the amount that is going to be left in your product.

Generally this occurs when you are further on in development, and this might be prior to licensure. There was some conversation earlier of whether you could drop having a specification at Phase III. The counter-comment that Wassim [Nashabeh, Genentech] made was then how do you know how much impurity you had in your final product that you used for commercial testing, where these are the clinical trials that tell you the true levels of safety and efficacy. I would agree with the conclusion that I hope was coming up there, which was that pre-Phase III is not realistic. It is probably going to be more pre-licensure, if at all, and that still depends on your manufacturing history.

The final reason for specific HCP assays is to help you with your evaluation of **product immunogenicity**. As you all know, the agency is very interested in having you determine what the immunogenicity of your product is. And if you find that you are getting immunogenicity, using an ELISA for example, you will need to know whether the immunogenicity was against the host cell proteins, or whether it was against your product. We have had cases, particularly where products are made in Pichia (Pichia proteins are very highly immunogenic in humans) where we have apparent immunogenicity to a product, but then when you tease it out by doing Western blots and figuring out what the different bands are, it turns out that that immunogenicity was to Pichia and not to the protein of interest. So if you have a good host cell protein assay you'll be able to tell between true immunogenicity to you your product and whether the immunogenicity is tangential and possibly of no consequence.

You also need to be reducing the amount of host cell proteins in your product in general in order to reduce the potential for allergic-type reactions and anaphylactoid-type reactions. There have been a couple of cases where there is a suspicion that there has been a reaction to host cell proteins. There have been a number of cases of anaphylactoid-type reactions and there has been concern that it actually might be due to host cell contaminant in the product and not to the product itself. Similarly, there has been difficulty in distinguishing what the immunogenicity is against. So the more you can reduce your host cell proteins, the less concern we have about this. In this regard, it makes a big difference if you are giving one acute dose of your product as you might do for a thrombolytic, or if you have a product that is being used to treat a chronic disease like cancer where you may be dosing many times. Those are factors that go into determining just how important it is to reduce your host cell protein levels.

And then finally there have also been some concerns that if you have residual host cell proteins, for example from bacteria, that these might have an adjuvant effect – meaning that the patient receiving it recognizes that there is foreign protein coming into their bodies, the immune system is beefed up, and lo and behold you are also getting more antibodies against your product just because you have beefed up the immune system and its surveillance looking for foreign proteins. There have been some instances where there has been concern that there might be adjuvant effects of residual host cell proteins. Again, the reasoning for reducing the amount of host cell proteins that you have in your product: it is helpful, as much as anything, to help you sort out what problems you are having, and you need to be able to quantify HCPs in order to be able to do that.

TYPES OF HCP ASSAYS

Now to the different types of host cell protein assays. This will be one of the major topics I think we have for discussion: ● You can have an in-house assay, where if it is an immunoassay, you have developed your own antibody, or ● you can try to buy a commercial assay, and we would call this sort of the overarching generic route as opposed to the in house generic route. So I guess there are two generics and we are going to have to be very careful with our terminology. But I am talking about the overarching one where you could go to a company and say, 'I need a protein assay for *E. coli*.' So then they give you an *E. coli* kit. And I know that many of you are looking into this. That is the generic that I am referring to as opposed to the in-house type of generic.

With the **in-house host cell protein assay**, there are a couple of ways that you can go about those. If it is an immunoassay you can make a host cell extract and put that into a rabbit or a goat to get a polyclonal antibody, and then look for the spectrum of proteins that you will see in your starting product, and along downstream purification. And as the two speakers following me, Kathleen [Champion, Genentech] and Helena [Madden, Biogen Idec] will talk about, there are advantages and disadvantages to what antibody you are using and how you got at that antibody, and what are the antibodies even seeing. So I won't talk about that too much because their talks will go into that in detail. But one alternative approach would be, for example, to take the first fractionation step of your purification process.

In this case, you have removed maybe let's say half or 60% of the host cell proteins that you have. The advantage of that would be that now you are zooming in on those proteins that come from the host cell that really are going to be part of your manufacturing process. And so you have a greater likelihood, if you then inject that to make antibodies, of getting antibodies to those species versus if you are looking at the total host cell extract. There may be a few really highly immunogenic proteins in there that are going to direct the immune reaction, but those proteins aren't really necessarily the ones that are going to be in your process.

The disadvantage of using a first fractionation step is if you have variability in that first fractionation step, then which first fractionation step do you use to generate the antibody? And so, as for everything, there are pros and cons, but in the agency we are certainly willing to look at both of those possibilities.

And then there is the question of having a process-specific versus generic [assay]. I won't talk about that too much because I think Helena [Madden] is going to talk about that more in her talk. But what this is referring to is basically: can you make a host cell protein assay to E. coli, assuming that all the E. coli's are the same? Or do you have to have one that has all the molecular changes that you have put into your production strain before you start to use it to make your product and then just focus in on that? If you want to use the former, then you are going to have to verify the suitability of that assay.

For in-house assays, in terms of what the assays might be: You have ELISAs. The advantage of an ELISA is you can get a quantitative number because you have a spot or a well and you are only going to get one number coming out of it. This is where the numbers of ppms generally come from. In the agency we generally see ranges of about 0.5 to 100 ppm occurring in our final products. And again, that's nanograms per milligram product. So it gives you a lump number – something that you can quantify.

One alternative is a Western blot. I happen to be personally partial to the Western blot approach because even though it is not as quantitative, a Western blot can be semi-quantitative, especially if you titrate the load that you have. You quantify how much protein you are putting on the gel and then you titrate it out to see at what point are you losing detection of various of the bands. But the reason why I tend to be partial to the Western blot is because it gives you so much more information and informs you of the complexity of the host cell proteins that you have in your product, and it helps you if you need to identify what are some of those contaminants if you have any major contaminants that co-purify with your product.

The specification for a Western blot is not fully quantitative because, well, which band are we quantifying? But is usually something like 'conforms to reference'. And this is a very subjective readout. So we are balancing the power of the technique – and again, we will hear some differing opinions about which is better or not, but this is my own personal opinion from my own experience of it – you are balancing between some difficulties in defining the specification and having a little more mush in there versus being able to get a number, because we like numbers and we like to be able to quantify and compare.

And then there are other approaches that Kathleen [Champion] is going to show you – some really beautiful work that is being carried out at Genentech. And that is using a proteomics approach to analyzing your host cell proteins.

What about the **commercial or generic type HCP assays**? This is where you are buying, for example, from a company like Cygnus. Well obviously these assays are host cell specific, so you say "I want an *E. coli* kit." They are usually for ELISA-based assays if I am not mistaken – and if I am, please somebody correct me.

Everybody wants to know, "Can we use one of these commercial generic assays?" In principle, you can. But you have to show that the assay is doing what you think its doing. So you need to validate the suitability of its use for your production strain, and that it has the appropriate sensitivity.

We did have one case where a manufacturer was using a commercial kit for Pichia and they found that no matter what they did they were having about 30 nanograms/mg of host cell proteins in their product. They realized, "Huh – I wonder if this is really doing what it is supposed to be doing?" So they did exactly the right thing – they made their own antibody to look to see what their residual host cell proteins were, and lo and behold, they had levels in the thousands of ppms of host cell proteins remaining in their product. It turned out that the commercial assay wasn't picking up even a hundredth of what they had in there. So this sponsor did exactly the right thing. They realized that there was some problem here. They went and tested it, figured it out, and realized what they needed to do in order to be able to solve that problem – make an in-house HCP assay.

So, our experience to date with the commercial assays is not ideal. Part of this is because it may have low sensitivity, and part of it is you really have to assess the suitability of the assay for your cells.

In the case that I just related, the Pichia strain that was used for the commercial assay and for product started from the same source type of Pichia, but the sponsor had introduced a couple of mutations in order to get their production strain. And it might be that that led to the differences, or it might be some lack of adequate testing of the commercial products in order to establish a good sensitivity. But if you want to be able to use one of those assays you are going to need to take a sample of your extract, take a sample of the commercial extract, and compare the sensitivities of what you are seeing. Do the bands relate? Is there any correlation between what that antibody can detect and what the proteins are in your product? This goes to make the point that if you are using an immunoassay, the quality of the assay is determined by the quality of the antibody. So we do need for you to qualify those antibodies and tell us about their specificity, and their sensitivity. What did you use to get it, and how have you tested it to let us know what its properties are?

There is one advantage to having a commercial assay – and this goes to the conversation that we were having this morning – that we would be able to do some cross-comparisons. "How much host cell proteins is Amgen getting?" "Well I don't know. Did you see Wyeth's numbers?" "Well, at Genentech, now they are down to 1 ppm." We would really like to know, you know, what kinds of levels are occurring in the different products. It would really help us to be able to get some assessment of the safety levels that we can tolerate. But, we are no where near being able to do that yet. If there were a way to systematize this and get some quality control on it, there would certainly be some benefits. Although I can see how companies might not want to have that because then they have to compare 1 to 10 ppms, and they have a press release that says, "Oh, we only have 10 ppms. They have got 100 ppms." But since we don't have generic biologics yet, that maybe won't be such a big problem.

SETTING SPECIFICATIONS

And now to the question of setting specifications. This is one of the major topics of conversation, and probably one of the harder issues for us to come up with hardcore directions that we can give you. So I can just tell you some of the factors that go into how we determine what a specification should be:

- Those relate to, very importantly, your manufacturing capability and your manufacturing history. As we were saying earlier: How much can you reduce the host cell proteins in your product? And how much can you do this reproducibly? Because if you can routinely get down to 10 ppm in your process, why would you have a specification of 1000 ppm?

- It also relates to your ability to characterize your product. If you have means to characterize your active ingredients, your API [active pharmaceutical ingredients] – then we can tease out what the host cell protein contribution is compared to what your protein of interest is. So the better you can characterize your product, the less we have to worry about what the host cell proteins are, provided that we know that they are host cell proteins and not product-related variants. That goes into our decision making.

- The safety profile of the products is from everything that you have learned, both from your preclinical studies and your early safety studies.

- Importantly, the dose – the amount of drug that you are putting in and the dose schedule. If you are doing repeat dosing, you are just as likely to increase your immunogenicity to your product as you are to your host cell proteins. So if you are doing repeat dosing, that is a concern. And how much? As I was listening to the discussion this morning I was thinking, a ppm really isn't a meaningful number if you don't know the dose of your product. As reviewers we are constantly calculating, "OK so they have, for example, a limit of 1 e.u. of endotoxin per milligram, but if you are going to be injecting 10 grams per dose, you actually may go over the limit of the amount of allowable endotoxin. So I am wondering if we shouldn't actually have a readout which is 'amount per dose.' Because if you are delivering let's say 100 ppm, and you are giving 100 mg of product versus if you are giving 10 micrograms of product, there is a huge difference in terms of how we will gauge what the importance is of the contaminant.

- The cell source makes a difference. Except for the instances that I told you about where there have been some concerns about either possible anaphylactoic reactions or possible adjuvant types of effects, we don't really have any information that one cell source is any worse than any other. There have been concerns, for example, about yeast because many people have yeast allergies. But it doesn't seem to be panning out with the levels of protein that we are talking about that this is really an urgent problem.

- The identification of the major contaminants: If we know what they are we can know how much we need to be concerned about them.

- And then, as I had said earlier, the minimization of any potential immune response to your product.

ADDITIONAL POINTS

There are a couple of other additional points to make. One is that there have not been many adverse events caused by host cell proteins. Consequently, in the agency, HCPs are not something we are particularly heebie-jeebied about. It is important to measure the host cell proteins for the reasons that I gave, but this is not an area where we have our greatest concerns. This is just to give you a little bit of a sense of where we are with that as an agency.

You should be using your host cell assay to **minimize impurities**. And this is to limit the potential for adverse events and to decrease any potential immune response to the product. You can imagine an example of molecular mimicry where if you have a bacterial product in there and there are some epitopes that are similar to a protein that we have endogenously, and the immune system says, "Aha! That is an epitope – I need to go after." But then it turns out that they go after the protein in you, you have an autoimmune effect. We have no known instances of that, but given the role of pharmacogenomics, it could be a concern. Of course we want to reduce any toxins.

Also, extremely importantly, you need to **validate your assay**. This is probably one of the strongest reasons to have a good host cell protein assay. So that we will know reproducibly what your process generates in the product, because lot to lot consistency, as you know, is of utmost importance.

One more piece of advice – we would recommend as an agency that you develop your host cell protein assay as early in **development** as possible. There was some talk earlier about whether you should start with a commercial assay and then move on to an in-house assay as you move along in development, and I realize that for smaller biotech companies this might seem cost effective. But the converse side is, first of all, that assay still has to be validated to show that it is going to detect the host cell proteins in your production process. Also, you will not be developing any relevant manufacturing history about what you really can accomplish. If you are using the same basic assay throughout, then you can come to us with your license application and say, "we have run ten production runs of this material and we consistently have less than 10 ppm" versus, "well, we had an earlier assay before but we are not really quite sure of the sensitivity, but for these last three runs we had this in house assay." You can see we really don't have as much information to go on in terms of making a determination for the specification.

It will also hinge on product **characterization.** You will know what are your product-related versus your host cell protein-related bands or peaks when you are utilizing a good HCP assay.

Having a good HCP assay early in development will also enhance your flexibility in making manufacturing changes. This is probably going to be very important to manufacturers as well. If you know how much host cell protein you are removing out of each step and you change that step and you can measure and say "aha, well I didn't change my host cell protein levels when I changed the process," well then that is one parameter that says "fine, we can make that change and we don't have to worry about comparability issues from that perspective." So it helps give us some information and flexibility.

Finally, the most important advice is to demonstrate that the assay is suitable for its intended purpose. And that just goes to say, if you want to show how much host cell protein you have or if you want to show you are removing them, make sure that the assay is really doing that.

Case Studies Reviewed On Product-Related Impurities

The consideration of product-related impurities on the second day of the forum began with two presentations outlining industry and FDA perspectives on the issues involved.

CDER's Clouse represented FDA, while Renee Boerner from Diosynth Biotechnology provided insight from industry. They were followed by Dieter Schmalzing, a quality control specialist at Genentech, and Laura Bass, from Pfizer, who presented case studies.

➤ Clouse gave a broad introduction to product-related impurities issues by defining the topic in relation to the process-related impurities discussion held the day before. "We can have process-related impurities," she said, "but there are also product-related impurities that arise from the basis of general product heterogeneity and also variants that may arrive during the manufacturing or storage process."

Clouse commented on FDA's interpretation of ICH Q6B's guidance on variant characterization, specification-setting, and "critical" and "non-critical" assessments during the application period (*see box, pp. 25-27*).

Boerner supplied the conference with a catalogue of some of the individual impurities that can complicate drug development, including adducts, aggregates, amino acid substitutions, carbamylation, fusion proteins, cleavages or truncations, deamidation, disulfide formation or scrambling, glycosylation, oxidation, PEGylation, and post-translational modifications.

"I think we are very good at identifying product-related variants and characterizing them," she assessed. "The thing that we are not so good on is identifying the impact of the product-related variants, and impact on safety and efficacy."

Schmalzing and Bass explained how their companies looked for, identified, and eventually handled product-related impurities during the development of recent products.

Pfizer's Somavert Presented Analytical Challenges

Bass discussed some of the challenges Pfizer encountered during the development and registration of ***Somavert***, the firm's version of pegvisomant for injection for the treatment of acromegaly in patients with negative responses to surgery and/or radiation therapy.

The Somavert project was initiated by Sensus Drug Development Corporation and continued after the San Antonio, Texas-based firm was acquired by Pharmacia in 2001. Pfizer took over development during the initial phases of its merger with Pharmacia in 2002 and saw the biopharmaceutical to approval in early 2003.

Bass explained the strategy employed with the growth hormone receptor antagonist B2036, a protein composed of 191 amino acids. "There are many sites that are susceptible to chemical modification," she said, "and it is during development that we identified each site. We evaluated the susceptibility – the extent to which the sites were modified – and we developed the tools to monitor these modifications and ensure we were controlling these modifications." Bass noted that the molecule was PEGylated in nine potential locations, adding a "whole other layer of complexity" to the process.

The polyethylene glycol (PEG) group used was capped with methoxy groups that prevented the PEGs from cross-linking, which can lead to aggregation or deamidation. Bass described the PEG as increasing the hydrodynamic volume by forming a "polymer cloud" around the molecule. The enhanced size of the structure, however, hindered the development team's ability to monitor changes to the biomolecule, the linker, and the PEG group itself.

Pfizer inherited the project in the later stages of clinical development, and as expected in these types of transfers, Bass explained, uncovered several gaps in the information relating to the process and product: ● the process development was incomplete with regard to yield, cost, and quality of the materials ● the analytical characterization was inadequate for a Phase II molecule; critical and non-critical process parameters had yet to be clearly defined ● the drug product formulation raised concerns ● and there was a need for process qualification and validation.

➤ FDA responded in June 2001 to the NDA submitted by Sensus in 2000. The action letter received by Pharmacia detailed 16 CMC deficiencies that needed to be addressed.

"We requested a teleconference," Bass said, "which was issued, and it focused on very limited technical issues – scientist to scientist – but it opened the door for discussions on the proper path forward." Pharmacia agreed to correct the agency's concerns and re-file its

application 12-18 months later. At that point, an "extensive investigation" was launched into the development of the process and the analytical methods, and a "thorough characterization" done of the molecule before and after PEGylation.

The first goal was to improve the analytical methods for evaluating the product's PEGylation profile. According to Bass, the Somavert team eventually began to use SDS-PAGE to "ensure we got the right extent of PEGylation in our product" during pooling and then used capillary electrophoresis to establish tighter control later in the process. Ion-exchange chromatography was used to isolate each individual PEG species and then a Malditov test was used to determine the mass. SDS-PAGE and capillary electrophoresis were used once again to double check the purity.

Pharmacia/Pfizer began to experiment with a variety of assay methods to address the characterization issues created by the protein's complexity.

Among FDA's requests was that an assay be developed to monitor oxidation. Kinetic experiments performed with the aid of a tryptic map confirmed where the oxidation "hot spots" were in the protein's structure. The map "focused on only one site of oxidation," Bass explained, "and we were able to quantitate oxidation and validate this method to support our program." The tryptic map is also used "as a supplemental method to confirm the consistency in sites of PEGylation....We were able to follow the decrease in peak areas and actually come up with a range of PEGylation for each of the predicted sites."

The agency also requested that the overall purity of the product be enhanced. "Our goal was to develop and validate a robust manufacturing process to produce API with greater than 90% purity," commented Bass.

"Based on the levels of des Phe trisulfide and aggregates seen, we targeted reduction in these variants and we used small-scale experimentation to identify where these variants were formed in the process, what the process did with them, where they were removed, and by what mechanism they were formed. We implemented process changes, scaled them up, and demonstrated by piloting it at full plant scale." The resulting process changes included additional manufacturing steps, resin replacements, changes to the peak collection strategy, and the implementation of capillary electrophoresis.

➤ Bass contended that "the real challenge" in understanding the product and its impurities "is addressing safety, and we all know that evaluation of safety is hindered by the lack of appropriate immunogenicity models."

Toxicology data derived from a variety of animal models was used with Somavert as a clinical study safety predictor, she commented, "but we all know that clinical data is the key indicator of safety and it is that clinical data that provides the link to the product quality profile."

The Pfizer official stressed the importance of retaining both initial toxicology information and the clinical study results for use as comparative sources to assess immunogenicity concerns. "In the beginning, our analytical methods are often deficient, so we do not know all the impurities present. So not only are your analytical methods changing, your process is changing. At each milestone, you need to go back to the beginning: look at the levels of variance you have, look at your tox data, look at your clinical data and try to make that link to ensure safety moving forward."

Bass considered a scenario in which capillary electrophoresis detects an impurity level that cannot be isolated. To evaluate the impurity the lab tries to produce an enriched sample containing, for example, 50% of the impurity. In such a case, she explained, "you have to consider the precision and accuracy of your potency model also and then you need to look at the degree of change. Was it 50% reduced, zero potency or was there only a slight decrease or slight increase in potency?"

➤ Going back to the levels of the variants present in toxicology and clinical studies provided Pfizer with data supporting the safety of its molecule as the firm moved through each phase of development.

In Somavert's case, des Phe trisulfide, N-succinyl, oxidation, and deamidation showed little change in potency and no signs of safety issues based on clinical data and were all designated product-related "substances" rather than "impurities." However, since the substances were seen "to be key indicators of product quality, process consistency and/or stability," Bass explained, "they were still monitored and controlled and specifications were set around these quality attributes."

Analytical methods were subsequently designed and validated with a low limit of quantification (LOQ) to support a tight control on these product-related variants.

Genentech Impurity Approach Outlined

Following Bass at the CaSSS meeting, Genentech's Schmalzing discussed his firm's "high level approach to specification-setting."

"First of all," Schmalzing explained, "we start out with the physicochemical characterization" to determine the desired product and its molecular variants. Included is an analysis of where the variant occurs structurally, how much is present, and how and where in the process it originates.

Genentech then classifies the variants into "impurities" and "substances" – a task that requires additional tools and information. "We then look from more of [a] behavioral way on our classified variants," Schmalzing said. "What is the safety, and efficacy, and the activity profile associated with those variants?" Much of this data is gathered from the preclinical and clinical studies, he noted, because the heterogeneity of the mixtures frequently makes determining the safety of individual variants difficult.

- There are two divergent approaches that may be applied to the safety assessment, Schmalzing commented: developing a "safety profile" of the individual variant – an approach that places a priority on product characterization; and choosing instead to gather "very little information" and to rely on post-market studies and putting "money toward possible adverse events."

In view of the "industrial regulatory climate," Genentech "might take a conservative approach with respect to our application" and establish an in-depth safety profile for the variant in question, Schmalzing noted. As an example, he described the discovery of aggregates and fragments during the initial physicochemical characterization of a product.

When these variants were found, Genentech assessed their activity using HPLC and iso-exclusion methods, which allowed the firm to isolate and place the enriched fragments and aggregates into a potency assay. "What we found here is that the reactivity was highly reduced for those aggregates and fragments," said Schmalzing.

A "direct safety signal," however, still did not exist in this case. As such, "because of the reduced activity and because of the regulatory industrial climate," explained Schmalzing, Genentech classified the aggregates and the fragments as product-related impurities.

- Genentech's procedure is to create a table listing all of the variants in the product as either product-related substances or product-related impurities. "We would also go then one step further and attach numbers, and percentages" for each of those variants, Schmalzing added, "and this table would then be included in our CTD [common technical document] filing in the impurity section."

The CTD addition becomes the starting point for determining if the impurity needs to be controlled. Genentech scrutinizes both a product's manufacturing consistency and stability data in both of their real-time and accelerated forms. If controls are deemed necessary, specifications are set according to preclinical, clinical and manufacturing data ranges.

In the case cited, "we found that the aggregation levels were consistently low," noted Schmalzing. "We [also] found no increase on stability. However, because it was classified as a product-related impurity, we would add it to our C of A [certificate of analysis] and we would essentially control for it."

Sometimes a safety profile is deemed unnecessary. As an example, Schamlzing described an occasion when oxidation was found on the methionines of a Genentech product. In this case, dose escalation was used to determine if the variant was a product-related impurity or product-related substance. The material was chemically oxidized to a level higher than the one used during manufacturing and then placed in a potency assay to assess the activity of the material.

The assay determined that there was no need to conduct a safety profile. "We found that the amount of oxidation we saw was consistently low," Schmalzing said. "In addition, we did not see an increase on the stability, so we did not feel in this case that we had to have a C of A testing performed on oxidation." However, Genentech did put in place "a validated back-up oxidation assay, in case there was any occurrence during manufacturing of oxidation, which would allow us to basically have a meaningful investigation of the oxidation event." He added that the

classification was later substantiated by the absence of negative effects in the clinic.

"Genentech's approach," Schmalzing concluded, "is fully embedded in Q6B, which clearly says that the specification should focus on the characterization, on the characteristics that ensure safety and efficacy of the product, and that we should have individual and collective acceptance criteria for product-related impurities and that the acceptance criteria should be based on preclinical and clinical experience, manufacturing consistency lots and stability data."

When Do Protein Variants Need Specifications?

Several themes from the process-related impurity debate on the first day of the CMC strategy forum carried over into the discussions of product-related impurities on the second day.

Participants continued to wrestle with the issues of whether standards were achievable, when specifications are required, and the implications of impurities on product safety and quality. The complexity and heterogeneity of proteins and their variants, however, further complicate the issues.

➤ During the discussion session, participants were asked to consider three central questions that companies have to wrestle with in developing a viable strategy for handling product-related impurities: ● What defines a product-related impurity and a product-related substance? ● How do you define a critical vs. non-critical product-related impurity? and ● What is the strategy for setting specifications for product-related impurities/substances?

At issue during the debate was how the significance of a variant is determined and how that significance relates to the need for a specification and/or increased control in the manufacturing process – concerns about which there has not yet been much formal regulatory guidance beyond the give and take between individual sponsors and the agency.

In looking for where some more universal principles and standards are achievable, the forum participants debated what types of variants need to be controlled by specifications, how many variants firms need to set specifications on, and when in the product development process they should be established.

A general consensus emerging from the debate was that determining whether a variant should be dealt with as a substance or an impurity hinged on the impact it had on the activity of a particular product and that it was difficult to set specification standards without considering the specific environment in which the variant was appearing.

➤ Within this complexity, participants explored the potential for sharing of their respective experience in a way that the more universal patterns could be understood and the wheel would not need to be reinvented for each new product.

The first part of the discussion centered around defining general principles for assessing specific impurities and establishing control standards on them. Later on, the main product-related impurities were listed in a grid format as a vehicle for surveying forum participant experience with their respective impacts on safety, efficacy and quality. Participants agreed that further work on such a grid would be valuable and should be pursued as a regulatory vehicle.

"Substances" vs. "Impurities"

Forum participants generally concurred with a comment made by Schmalzing that the decision to characterize variants into product-related "substances" or "impurities" will depend on the kind of variant and the available tools, and not on a specific protein concentration or what the final concentration will be for every dose.

Pfizer's Bass explained how the assays would assist the classification process. "If we know that this protein has methionines or asparagines that could be oxidized or deamidated, we would tend to go about the approach from a due diligence perspective....We would look for those things and we would attempt to improve our assays enough to detect charge heterogeneity or size heterogeneity, and then with those assays, go in and start with" those variants with a significant concentration, "maybe the 3%-5%, and confirm the identity of those."

➤ A consensus was voiced during the conference that a thorough molecular characterization should be undertaken to the extent possible during the early stages of product development, allowing the process to be better understood.

Genentech's Blank asserted that "the money that you spend on characterization, whether it is biological [or] structural," upfront results in a more "robust manufacturing process with regard to specifications [and] is money very, very well spent." FDA's Shacter agreed. "Rather than intentionally introduce modification that you know really will not be relevant for your process," she said, "know what variations you will have in your molecule as it is going through the clinical process. Then we know whether we need to be concerned about it or not."

Biogen Idec's Rohin Mhatre observed that firms are going to have to make regulatory decisions based on this characterization. Even if a variant is present at low levels, he said, "you may not want to specify, but we tend to have specifications for product-related impurities more so than a product-related substance that does not have any effect on safety, efficacy or bioavailability…You want to characterize these things if you can. Particularly if they are at a sufficient level to really impact potentially, you want to control it."

Also important during the early stages of development is construction of a reliable assay system. Genentech, said Schmalzing, is "really putting the meaningful assays in place" as early on as possible to minimize any kind of retest that could have to be done later.

➤ "The more relevant [the assay] is to the physiological activity of the molecule," said FDA's Shacter, "the more it helps us to determine whether something has an effect on the true function."

"Often the potency assays are in the process of evolving early in clinical development," observed FDA's Clouse. "So if you test in one particular potency assay early on – say a binding assay – and you lull yourself into believing that there is or is not an effect on potency, you may be surprised later when you develop additional biological assays that indicate a difference."

Clouse later added: "We often have or request both a binding assay and a potency assay….Although you would expect that the biological activity would fall off if there is a problem with manufacturing or with a product, we actually find that, in some instances, the binding assay is a greater indicator of stability. In others it may be the bio assay if the specifications are set tight enough…. You have to have a bio assay that is as much as possible deemed relevant to the mechanism of action for the product, with the caveat that you may not know for sure what the mechanism of action is, but at least you should have a general idea."

Spec Setting Is A Dynamic Process

Developing appropriate standards varies on a case-by-case basis and may change over time. "Specification-setting, I think, is a dynamic process," Schmalzing commented. "Once you have set specifications, it does not mean that those are being written or set in stone."

➤ Schmalzing briefly summarized Genentech's method for establishing specifications.

Just after the early product characterization, but before the product concentrations are fixed, an arbitrary action level is established around a target concentration that triggers an in-house investigation if breached during the manufacturing process. As the development of the process progresses, the action level is periodically narrowed to assess production consistency. The "provisional specifications" are then adjusted as preclinical and clinical data arrive to determine a safe and efficacious composition of the product and are eventually removed once consistency is established.

"To give *a priori* numbers, for example for a potency assay," Schmalzing elaborated, "maybe I might set a specification +/- 30%, +/- 40% around a target value. But this is just to ensure that the potency of my material is not dropping too low – so that I don't put something in the clinic with very low potency values. Then I would slowly tighten those numbers."

➤ The FDA officials on the discussion panel commented that there was not a percentage of variant concentration that marked whether a specification or further purification was needed.

"We don't have any hard and fast rules about how pure something has to be," said CDER's Shacter, adding that the most important figure the agency looks at is not a product's level of purity, but its manufacturing consistency. "If you have an impurity and you do not know its possible impact on safety or efficacy," she elaborated, "then you will need to probably have a specification for that element until you can generate some data for whether it makes a difference or not. And if you have data that shows that it does not have any effect on safety or efficacy, then we can loosen up on the requirement for having a specification. But until you know that, you probably need to specify it."

The flexibility the agency allows in establishing specifications also depends on the type of variant in question. "If you have deamidation," Shacter explained, "we are not particularly concerned. We just need to know something about it. But if you have aggregates, it is a whole other matter. So if there is some variant that we know has a greater likelihood of having an impact on safety then we will definitely ask you to...control it, and if possible decrease it."

"The whole purpose of setting specifications is to confirm the quality of the product," said CDER Division of Therapeutic Proteins Deputy Director Barry Cherney. "If you have a set of tests that you think are adequately designed to confirm the product quality, then that will be enough...When you get degradation patterns...and you see some attribute that degrades...we like to have that on both the specifications and the stability testing."

FDA's Clouse concurred: "My comment to a sponsor," she said, "is that, generally, you must have a point for every criterion you are evaluating for which you yourself, or the sponsor, would not want this to go into the clinic."

One participant advocated the use of a target potency instead of a minimum safety allowance. "I think if you essentially lower the standards and allow for [a] more or less pure, [or an] extremely less pure product early on in development, if you do have any type of adverse events it may prevent this product from going forward because of the inherent difficulties," the audience member said. "My feeling is, once you get ready to put it into the clinic, you should try to have something at least as well-characterized, or as clean, as you intend to license, because otherwise you will go back and if you have a lot of change, you may have to essentially reinvent the wheel and repeat some of the clinical studies because the product is so different."

➤ Cherney noted that product-related substances may not need to be specified, and that specifications included for approval may be sunsetted.

"Sometimes, you know, even at approval, if you have convinced us that a variant is actually a product-related substance that does not impact safety and efficacy," said Cherney, "we are not even going to ask you to specify it at the time of approval. Other times … as you get more information on that – you get more and more about product-consistency – you can sometimes be relieved of some of the tests that you are doing on specifications. I know...in certain instances, we have asked people to look at disulfide bridging, and things like that, early on, and then...later on, we release them from that specification. So as you are getting more knowledge on your product, as you are getting more consistency, you can get some relief from some of the tests."

Pfizer's Bass suggested that specification "sunsetting" may be a defining characteristic of non-critical variants. Conversely, variants considered to be critical to the product are those that require specifications throughout the manufacturing life-cycle.

While recognizing the potential for specification sunsetting, FDA officials warned against removing them from a variant too early in the process. "There really does have to be some clinical experience with the molecule, I think, before you can determine what the critical parameters are for some products," Shacter cautioned.

FDA Does Not Require Specs On All Variants

The number of variants that need to be specified for a given product was also examined during the forum discussion.

Schmalzing explained that Genentech's specification process is applicable to both product-related impurities and substances, and advocated specifying – or at the very least "monitoring" – all variants during development.

➤ Agency participants reaffirmed that establishing specifications for every variant that appears in a product is not required by FDA.

"We do not want to discourage you from actually characterizing your molecules, and we realize that specifications are really an odious element for sponsors because there are that many more parameters that could fail your product," Shacter affirmed. "You do not necessarily have to have a specification for every variant that you know of, or every modification that you know of, and then take it away over time." Clouse echoed Shacter in explaining that "we really do not want to discourage you from characterizing your molecules as thoroughly as possible, and we will enter discussions with you to figure out which parameters really do seem to be critical and which are not."

Despite the FDA flexibility, industry officials generally viewed a lack of specifications as a potential liability. "I think that is something that we always have to do," a

participant maintained. "If it is aggregates, or deamidation, or oxidation, if we are concerned about them increasing or we know that they are going to increase in the drug product then we absolutely must have specs on them for our business reasons."

In some instances, variants that do not need to be specified to capture potential changes in safety and efficacy can serve as barometers for other molecular characteristics of concern. "Case in point is oxidation," Cherney pointed out. "Although the oxidized methionine residue does not affect safety and efficacy, it is a stability-indicating assay in that it is a very good surrogate for one of the first things that happens to a molecule when it is under stress … But in lieu of knowing anything about that variant, you are going to tend to need to specify it if it is at a measurable level where you can really quantitate it."

CDER team leader Stephen Moore noted that ICH Q6B offers the potential to reduce the labor involved in specification setting by allowing for pooling of the various product-related impurities into one specification.

"That is something that I personally do not particularly like," one audience member responded, "because I like to get more information on each product attribute instead of pooling them all together." The participant said he was unaware of many manufacturers who actually used specification pooling during their processes.

- Amgen's Heather Simmerman made a distinction between end-product testing and in-process testing, suggesting that firms should evaluate "what really does need to be your product quality spec versus an in-process control for the manufacturing consistency and not put the strictly manufacturing consistency parameters on that end-product testing."

Repeating a key theme of the January strategy forum on specification setting ("The Gold Sheet" January 2004), Cherney emphasized that "there are drastic differences in terms of regulatory implication. Achieving an action limit means [an] in-house investigation, whereas an out-of-specification result involves a lot more work and submissions … So there is an avenue, potentially, for putting things as an in-process control, and when you fail that limit, you would do an investigation. Part of that may be an additional characterization that is above and beyond what you do for routine specs to actually fully characterize and confirm that product quality has not been impacted by the process."

CDER's Clouse On Product-Related Impurities

At the strategy forum, Kathleen Clouse, acting deputy director of CDER's Office of Biotechnology Products, discussed FDA's perspective on assessing and setting specifications for product-related impurities.

The structure for this morning's session will be I will l give a very, very brief introduction to the whole concept of product-related variants or product-related impurities, as opposed to process-related impurities. Renee [Boerner] will then present more detailed information with regard to the types of product-related variants you may encounter and how to test for those. And then will have two presentations of case studies that hopefully will provide even more detailed information as to how to tackle the issue of various types of product variants.

As you all learned from yesterday and you no doubt know if you are involved in the manufacture of biotechnology products, biological product characterization involves the determination of the product's physicochemical properties, the biological activities, the immunochemical properties, as well as an assessment of the product purity and impurities. And as we learned yesterday, we can have process-related impurities. But there are also product-related impurities that arise from the general product heterogeneity and also variants that may arrive during the manufacturing or storage process. Using appropriate techniques to determine all of these characteristics will enable you to establish relevant specifications to make sure your product remains consistent throughout the developmental stage.

In the ICH 6QB document, product-related substances are defined as those variants of the product that have properties that are comparable to those of the desired product and also have similar activity, efficacy and safety. In contrast, product-related impurities are defined as molecular variants of the product that arise during manufacturing or storage which do not have properties that are comparable to those of the desired product with respect to activity, efficacy, and safety.

Now the question is how do you define "comparable"? What do you test? As we brought up yesterday with regard to process-related impurities, do you arbitrarily start with anything that is greater than one percent of the product, or when do you test it? And these are all issues that we hope to discuss later on during the discussion session.

ASSESSMENT

Unfortunately there are no specific guidance documents that tell you how to deal with all product variants. However, some information can be obtained from the ICH Q6B document on setting specifications. But even that document does not provide anything specifically with regard to how to deal with each and every variant. What it does state is that individual and/or collective acceptance criteria for product-related substances and impurities should be set. But it is up to the manufacturer to define the heterogeneity pattern for the desired product and also to demonstrate consistency in the manufacturing process.

Once you encounter variants, it is inherent that you **characterize the product and its variants**. Ideally, it involves the identification of the variant, the determination of the biological activity, assessment of the PK profile, and also an assessment of the safety of the variant, including immunogenicity. Clearly this isn't possible early on, and it is also not possible later on even if you were to isolate a variant because you can not realistically check the impact of that variant individually by injecting into a patient. So although the characterization does not necessarily have to be done also for every variant that you encounter, the additional information that you obtain for variants that are present in significant quantities allows more flexibility in setting specifications. And in addition if you maintain manufacturing consistencies so that you keep the number of variants at a minimum and you also keep their concentration at a low concentration, you can alleviate the need to characterize all the variants.

Now the **critical parameters** identified in Q6B for setting specifications as product development proceeds are based on information from: relevant developmental data; also stability studies that give you an idea of what type of product degradation you may have and the type of variants you may encounter; the manufacturing history, in particular the consistency lots, to see how much control you have over the process and how much you can contain or control the generation of variants; also the lots that are used in preclinical studies; and most importantly, what is essentially considered the gold standard, are the lots used in clinical studies. And the lots used in clinical studies and have data on are those that you really want to rely on most for subsequent product development and setting and maintaining your specifications.

SPECIFICATIONS

So once you have some idea of the manufacturing consistency, you can begin to set specifications. As was mentioned yesterday, we actually prefer that you set specifications of some degree very early on in the manufacturing process, because not everything you manufacture will be acceptable and there may be some situations you would have during product development when it is very early for setting specifications that you know you would reject a product, you know you would not to even test this either in preclinical or clinical studies. So, once you have consistency of the manufacturing process you begin to set specifications. When you go into preclinical studies and clinical studies, that data can be mined to help establish the specifications in a more narrow range. And finally, if you do an in-depth characterization of the desired product and as many variants as are present in consistent amounts, this can help establish links between the quality assurance, the safety, and the efficacy of the product.

The last point that I would like to make as an introduction are lessons that have been learned, or we can also call this a reality check. As mentioned yesterday, the identification of critical quality attributes or setting definitive limits for a product may not, and generally is not, complete by the time of product approval. It really depends on how many lots have been manufactured and how much data you have to draw on.

And the next point: One of the presentations yesterday brought up the point that when you set specifications you have a desired range that you want to achieve. And you generally set limits on either side of that, and ideally you set them closely on either side. But in some cases you may set them very narrowly on one side of the range at the lower end, but allow a little more flexibility at the upper end, for example.

Well, in those situations, what you have to keep in mind is if you have broad specifications and you have differences within those specifications that arise as you make manufacturing changes, and these changes are still within specifications but they are outside of clinical experience that you have with that product, you still may have significant consequences when this material is used in the clinic because you have no data to draw from. So it is something to keep in mind as you proceed with product development.

The other thing to keep in mind is that **manufacturing changes** may result in unanticipated product-related variants for which the clinical impact is not known. And in some case, for example with regard to immunogenicity, if you are beyond the point of product development where you are actually doing your immunogenicity studies, you may not actually monitor this change and you may not have a way of assessing whether or not there is an impact on immunogenicity unless you have specific studies designed to address that issue.

Aggregation Impact And Assessment Tools Reviewed

The final session of the program focused on the implications of protein aggregation in biologic products, as well as approaches to measuring and minimizing aggregate levels. The major concerns associated with aggregates continue to be their potential to impact product potency and to trigger negative immune responses.

The forum discussions reflected those at an IBC "well-characterized biologics" meeting in November 2003, where FDA officials presented "agency perspectives" on detecting and assessing the effects of aggregates ("The Gold Sheet" January 2004).

Michelle Frazier-Jessen, a biologist in CDER's Division of Monoclonal Antibodies, discussed "approaches to measuring and minimizing product aggregates." In the presentation, she addressed FDA regulatory concerns, methods selection and validation, assessment issues, causes and specifications (*see box pp. 28-32*).

Frazier-Jessen commented that applicants have typically had aggregate levels below 5% during the IND phase. However, she clarified that "other ranges may be acceptable," depending on "how good of a job you have done characterizing your product," and its type, indication and route of administration."

Zahra Shahrokh, Director of Pharmaceutical and Analytical Development at Transkaryotic Therapies, and Centocor's Micheal Bond shared their respective company's experience with aggregates. Shahrokh described some of the techniques her firm is using to evaluate and quantify protein aggregates, such as size exclusion chromatography (what she referred to as the industry's "workhorse"), gel and capillary electrophoresis, ultracentrifugation, and light scattering. Bond then supplied the audience with the advantages and disadvantages of using these and other methods.

➢ The afternoon discussion session focused on issues relating to the quantification, validation and characterization of protein aggregates.

The forum was guided by six questions: ● "What are good methods for quantifying aggregates and how should we use them (e.g. lot release, comparability)?" ● "What are the best assay validation approaches for aggregates (How do we address the differences between methodologies or the instability of the purified aggregates to determine LOQ, etc?)" ● "What are the considerations in setting specifications for aggregates (various clinical phases, nonclinical experience, manufacturing experience, analytical capability, etc.)?" ● "Are all aggregates bad (e.g. immunogenic)?" ● "What is the real evidence for immunogenicity of aggregates?" and ● "Does immunogenicity of aggregates matter?"

An informal survey of the audience revealed size exclusion HPLC to be the most frequently used method to characterize aggregates.

Participants noted that the effectiveness of some methods and decisions around specification setting can depend on the solubility of the aggregate. For example, ultraviolet light scattering tests tend to be more applicable with insoluble aggregates, while size exclusion chromatography tests are more useful for soluble ones. It was also pointed out that aggregates are often operating in a dynamic equilibrium system between monomers, soluble aggregates and insoluble aggregates, complicating measurements aimed at quantifying them.

➢ Ensuring that the results collected from any sampling exercise are representative of the final product was the dominant concern of the assay validation discussion. Participants raised the point that the act of sampling and measuring itself perturbs the product.

In some cases, as one participant pointed out, validation is made difficult when isolating an aggregate is not possible. It was noted that in such circumstances, manufacturers must generate the aggregate chemically using heat or pH adjustments. Manufacturers then must develop experiments to demonstrate that the artificial aggregate is representative of the impurity in question.

CDER's Cherney emphasized the need for manufacturers to use multiple, orthogonal methods in measuring aggregates, pointing out that size exclusion HPLC has limitations despite its usefulness. He explained that aggregate content can change as samples run through size-exclusion columns, allowing weak interactions to be lost. Cherney noted that discordance between two methods measuring the same product can be problematic.

The problem of defining aggregates was another issue discussed. Participants pointed out that proteins often self-associate and exhibit a large range of dissociation constants, posing the question of the point at which a self-associated group of proteins become classified as an aggregate. Cherney commented that, when examining dissociating protein interactions and their implications to product quality, the product's indication and route of administration should be taken into account.

Other questions that can complicate protein aggregation assessments are whether the aggregates are linked covalently or noncovalently and whether the aggregates assemble reversibly or non-reversibly. ♦♦

CDER's Frazier-Jessen On Assessing Aggregates

At the July strategy forum, CDER Division of Monoclonal Antibodies biologist Michelle Frazier-Jessen gave a presentation on "Approaches to Measuring and Minimizing Product Aggregates." She addressed: ● regulatory concerns ● methods selection and validation ● assessment ● causes, and ● specifications.

I am very happy to come and present my experience and the experiences of my colleagues in regards to monoclonal antibody products and what we see with regards to aggregates. I do want to stress that these views are really my own or those of my colleagues. During the presentation they may not represent policy – the standard disclaimer....

As far as aggregation and biotechnology goes, I think I am speaking more towards the aggregates that we see as a result of the manufacturing process, and pretty much anything you guys do in manufacturing can affect the level of aggregates in your products. Likewise aggregates are often a common degradation pathway, so how these products are stored and their stability plays a role in this as well. In spite of I think our best efforts to maybe control aggregates through manufacturing and storage, we still have the issues of dealing with the intrinsic properties of the molecules, and this is very product specific. I guess that by this I mean that…you can have four antibody products that you make in the same CHO cell, the same construct background, and yet the very aspects of the construct that make it unique from the others are also the aspects that maybe lead to a high propensity for aggregation in that product.

REGULATORY CONCERNS

Our concerns from a regulatory standpoint are that usually what we see is that aggregation tends to alter the **potency** of the product. I think that most people would suspect that that would be a decrease in potency. But we do see an increase in the potency on occasion. I think that is especially seen in instances where we might have an antibody targeted against a cell receptor and you get cross-linking, and then you get an increased bioactivity. Where this comes into concern is when we talk about the bioavailability of the product. If you are dealing with a product that has a very narrow therapeutic window then this is of concern as far as safety and efficacy go, because this can affect your PK. You could fail a trial for efficacy because your dosing schedule is not correct.

I think also the big thing that we worry about and that everybody talks about as far as aggregates is the potentially for **immunogenicity**. With regards to immunogenicity, what we could have is a product that if you get antibodies developed against the product, we could affect the bioavailability of the product as far as the PK because we have less drug available – it is being cleared faster. Or, worst case, we could have the development of an immune response to an endogenous protein, as we have seen with EPO.

When I was preparing for this talk one of the things that we were talking about within the group was what is the real case for aggregates and immunogenicity? Where does that comes from? I think that, at least in my review of the literature and in talking with other folks, there is one incidence that we are aware of where an increase in aggregation was associated with an immunogenic response. There is a strong linkage between the aggregates, maybe in manufacturing changes or things like that, that have been linked to the development of immunogenicity.

I think that we cannot put aside the fact of all the years of research looking at the responses of aggregates, even in the *in vitro* studies, when you stimulate an antigen-presenting cell with aggregates, you can induce T cells to become activated, and they are going to then stimulate B cells, and B cells are then going to produce antibodies. Likewise, you could just have a direct effect of just stimulating B cells and producing antibodies as well. I think that there is just a tremendous amount of data out there that we can't ignore that – that we know that this is a potential risk. When we are talking about these therapeutic biologics, if we have aggregates, we could be inducing and taking a protein that would normally not be immunogenic and by injecting it in an aggregated form we could be making that protein immunogenic or a product that is also weakly immunogenic we could make it more immunogenic.

METHODS SELECTION AND VALIDATION

So therefore, we, of course, feel it is very important that there are methods in place to monitor aggregates. The methodologies that a sponsor selects is really up to them. It is product dependent. It also depends upon the purpose. If you are doing a characterization of a product or a reference standard, or you are making some changes and you need to establish comparability, you might be doing a wider assortment of methods to look at your aggregate levels versus if you are just doing a day-in and day-out lot release. So that is an important thing to think about when you are choosing a methodology – what is it you are trying to get out of it.

The other thing too is that folks need to think about having complementary methods. Maybe you have your SEC as your main workhorse, but you have got SDS-PAGE as well that you can get some information with that might help you in detecting things that maybe your SEC is not going to pick up. So that is very useful.

These are the kinds of methodologies that I have seen for detection of aggregates in submissions, both INDs and BLAs: Light obscuration for particulates is really for particulates. SDS-Page, we see. We see CE [capillary electrophoresis]. And size exclusion [HPLC] is really, as was stated, the workhorse. Those tend to be the more the day-to-day lot release-type assays that we see. What we tend to see is for the other types of methodologies, they tend to be used more for comparability or general characterization of a product, or they can be used, as was stated before, to validate the other methods.

How you **validate those methods** is really going to depend upon, as was stated before – this is kind of going around in a circle, I know – what your purpose is. Some methods are not going to be very easy to validate, but you may not need to do that in that capacity because you are using it to establish comparability or to qualify another assay that you are going to.

But once you select the methodologies that you are going to use, and I think that especially for lot release and if you are going to do something that would be considered different or novel, you need to provide a rationale for that and you need to be able to justify it through data. Of course, ICH Q2A and Q2B are very helpful in determining how to do your validation. But they should not be considered just a checkbox. I think you really need to think about what it is you are doing and design things in that manner.

ASSESSMENT

I like to see that aggregates are assessed during the characterization, both initially and also by the time you get to the BLA you should have a really good understanding of your aggregates and any changes that you have made from manufacturing. You should have an idea of how that is affecting your aggregate profile, especially if you want to come back later with comparability for a new methods change. They definitely need to be part of your release criteria and they need to be part of your stability protocol.

This was another question that was asked of me: what **levels** are really appropriate? I think that it just depends. It depends upon a couple of things. The one that is really important and the sponsor's job is the characterization of the components themselves. The more thorough job that you do throughout your development of characterizing your aggregates: Are they reversible? Are they irreversible? This manufacturing change caused an increase – those kinds of pieces of information when you get to a BLA are extremely useful because it tells a story about your product and it shows that you really understand your product and how it is holding up.

Once you do that thorough characterization, then the second part, the assessment is kind of our job. But we can't do a thorough assessment if we don't have that good characterization, and that will effect our decision. Including the characterization part, we have also got to look at the indication, how are you dosing and how frequently, and that will all play into the decision of what we feel is acceptable, and if you have something that is different from the norm – these kings of things will help us come to some kind of a decision.

Typically, specifications for aggregates should not be more than 5% during the IND phase. By the time a product gets to licensure, these specifications will likely be tightened. Having said that, other ranges may be acceptable and that really depends upon, once again, how good of a job have you done characterizing your product, how thoroughly do you understand your product, and what your experiences are, not only just manufacturing but preclinical, clinical experience – what do you know about your product, its stability profile, in accelerated and real-time. Then, once again, if it is going to be different from the 5%, you are going to need to justify it. And if you have all this information you should be able to do so.

CAUSES

This has been gone through before, but these are things that we know cause aggregation: temperature, light, agitation, and formulation and container closure actually are a couple of others. I just wanted to talk about the last two, because I think that the other three we all know about. Having said that though, I think that a combination of any of these factors can affect the degree of aggregation you can get.

Touching on **formulation**, I think that it really behooves a sponsor to look at different formulations during their development process….This is actually a slide of a monoclonal antibody that has been formulated in different buffers and then put under accelerated stability conditions, and this is an ultra-analytical centrifugation study. What you can see is that not all formulations are created equal. This is very important piece of data as you are going through to know that to help minimize your aggregates. And certainly we also see sponsors putting in other surfactants and things like that to help control that as well.

Regarding **container closure systems**, every product has a different container closure system, usually. Traditionally we see glass vials – the type 1 borosilicate, everybody knows about that and that is what is probably the standard for a lot of these products. But we also see lyophilized products in kits, and we are starting to see a lot of pre-filled syringes. We might also see other formats, we don't know yet. But I think that we are so used to dealing with the glass vials that, when we are talking about, for example, pre-filled syringes, we do not really have as much information as to how aggregates are going to be formed in the context of this different container closure system, because we have got to deal with leachables and extractables as well, and they can all influence what we see.

So it is really important, if you think you are going to go to an alternative container closure system, that you have stability data in your to-be-marketed format. I do not think I could stress that enough, because if you look at Q1A it says you have to have stability data in a container closure system that represents your marketed format.

And I don't know how right now we can better represent a pre-filled syringe than a pre-filled syringe. I think that is really important for people to think about – that you need start looking at that and how your product is going to hold up under that. We have started to see some instances where there might be issues with aggregates or particulates forming in these syringes. I guess, also, I should probably mention, just as an aside, when you put your product into that I.V. bag if you are giving it intravenously, sometimes that can affect the level of aggregates – the I.V. bag that you are using, the solution that you are using.

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What we need so far as aggregates goes: we need to have a good **stability program** that has the intended storage conditions, and you have established that by real time and accelerated data, and then you have a profile of your aggregates. That can help you to set your specifications.

SPECIFICATIONS

Somebody asked me, 'how do you set specifications for aggregates?' Well, if we look to Q6B, specifications are a list tests, references to analytical procedures, and appropriate acceptance criteria. They are there to confirm that we have a safe and efficacious product and that it is of good quality. It is the company's job to propose and justify those specifications and then we approve them.

They are going to be very product specific. So once again, it depends – that is my D-word, it depends. It might be that if you have done this thorough characterization of your product, you find that you have reversible and irreversible aggregates, and you maybe need to have specifications for both of those. And if you can justify that, it would be something feasible. But you would need to have the data and to have a good understanding, and there would have to be a good understanding on our part as far as the potential safety issues with that. This really goes into the fact that your specifications are really going to be linked to your manufacturing process and all the studies that you have done and the procedures that you are using to measure the aggregates by.

They should account, also, for the stability of your product. So you need to be thinking about: What is my expiration date two years out? How is my product going to hold up under those conditions? I need to think about that when I am setting my specifications.

Finally, with regards to that, there is also the potential to have **shelf life limits versus release limits**. Q6B states that this is a concept that pertains to the establishment of limits that are tighter for release than for shelf life. I know this is very popular in Europe – that a lot of products in Europe have a lot of release limits and shelf life limits. And we are starting to see sponsors come in and ask us about those, and we are having discussions about them. They can be applied where they are justified. Once again, a lot of times you can think of release limits as more of an in-house limit and then the shelf life limit is how you expect your product to behave in its intended storage conditions for its expiry dating. But I do not think that shelf life limits are appropriate if there is a concern regarding safety and efficacy of the product.

So with aggregation, that is really going to depend if that is okay to have a shelf life spec, once again, on your product characterization. Are these reversible aggregates or irreversible aggregates? I think that that is really going to depend for those types of situations. But the more data that you have and the more thorough characterization that you have, it goes a long way towards making us feel comfortable.

Finally, I guess I really just want to emphasis in **summary** that the first and the last point is that we really feel that it is important to monitor aggregation because of the potential effects on safety, efficacy and the pharmacokinetics of the products. I think that the most important thing to do, as I have said over and over again, is really understanding your product and doing a thorough job on characterizing your aggregation profile. The work that you do is going to influence the decisions that we make with regards to the different components.