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CPMP POSITION STATEMENT ON DNA AND HOST CELL PROTEINS (HCP) IMPURITIES, ROUTINE TESTING VERSUS VALIDATION STUDIES

INTRODUCTION

Biotechnology products, in many cases recombinant proteins, are derived from a complex expression/production system involving a genetically modified host cell (bacteria, yeast or mammalian cell). Among impurities to be eliminated during the downstream purification process, two components which are of major interest for safety and tolerance reasons are **residual host cell DNA** and **residual host cell proteins (HCP)**.

Since submission of the first marketing applications for recombinant proteins, depending mainly on the type of production cell system, the European regulatory authorities have adopted different approaches to deal with these impurities. In summary, for HCP, whatever the product and production system, residual HCP have to be tested for on a routine basis, whereas residual DNA is tested for on a routine basis only for products derived from continuous mammalian cells.

It is necessary to guarantee that such impurities are reduced to an acceptable level in the medicinal product to be administered to the patient. For that, two approaches can be envisaged :

- a “validation approach”: to validate the production process to establish that, at given steps of the purification scheme, those impurities are removed in a consistent and reproducible manner to an acceptable level. Based on the reduction factors it may be possible to predict and guarantee the residual level of impurity in the final product.
- a “routine approach”: to develop analytical tools that allow monitoring, as closely as possible, of the level of those impurities at various steps of the process and set fixed limits to be met, so that the impurities are well monitored in the final product.

A combination of these two approaches is also possible, i.e. routine testing at an earlier step of the purification process and demonstrated reduction by validation in order to ensure a limit at the final product level, if tested.

In the context of the world-wide use of recombinant proteins, it is necessary to harmonise the evaluation criteria between the three regions of USA, EC and Japan, so that manufacturers can develop their products on the same lines whatever the intended regions for submission and marketing.

RESIDUAL HOST CELL DNA

Regarding **residual DNA**, it is already accepted for bacteria- and yeast- derived products that there is no need for routine testing provided that acceptable levels in the final product are achieved and, adequate validation data are submitted in the dossier. As far as DNA from continuous mammalian cell lines (CCLs) is concerned, this impurity was considered, in the past, as a risk factor because of concerns that residual host DNA may be tumorigenic. Further information, however, now suggests that CCL DNA poses much less of a risk than previously thought and accordingly should be considered as a general impurity (WHO Expert Committee on Biological Standardisation: Highlights of the 46th meeting, October 1996, in WHO Weekly Epidemiological Record, 1997, 72, 141-145).

Validation studies (e.g. spiking experiments using an adequate size distribution of DNA) should be performed in an attempt to identify the major steps capable of reducing the DNA burden and to document the capacity of those steps in reducing residual cellular DNA content in the final

product, to an acceptable and defined level. The technology for DNA quantitation is now well defined and reproducible.

In addition to the validation studies, results of DNA quantitation on a minimum number of production batches (e.g. 5 consecutive batches) should be provided to demonstrate the reproducibility of the production process in reducing residual DNA to the level expected from the validation studies. Based on satisfactory validation data and consistent results on a limited number of production batches, it seems reasonable not to perform routinely CCL DNA tests at the purified bulk level (or other appropriate steps).

In some cases (e.g. previously non-approved CCLs, transforming DNA sequences from viral vectors, etc.) it may be necessary to routinely control the elimination of DNA.

As far as products derived from CCLs already authorised are concerned, it could be acceptable that routine DNA testing be discontinued. However, this will be subject to a formal variation of the Marketing Authorisation. The variation documentation should provide satisfactory validation data, along with results gained by the applicant since the beginning of the production to establish consistency of the process, in terms of residual DNA level.

RESIDUAL HOST CELL PROTEINS (HCP)

The question of **residual host cell proteins** (HCP) concerns all expression/production systems and not only the mammalian cell production system. Indeed, for all recombinant proteins, the presence of such impurities raises the question of potential immunogenicity of the product. As such, it is currently required that HCP be routinely monitored at the purified bulk level, using suitable analytical assays. Results from batch to batch should be consistent and meet specification limits.

Regarding the acceptable limits to be set, it should be stressed that it is impossible to set a common limit of HCP contamination for all biotechnology products. Indeed, host cell proteins are impurities that vary qualitatively and quantitatively from one product to another and even from one production/purification system to another. In the same manner, standardisation of the analytical methods would be problematic as the reagents used in the tests are product- and production system-related. For HCP, it is difficult to identify the material sufficiently representative of the impurities to be followed at the relevant steps or to be used in a validation approach ("spiked material").

Finally, elimination of HCPs, in most cases, makes use of chromatographic columns for which the selectivity and yield of the procedures depend not only on the quality of the material but also on the way the columns are used and re-used, storage conditions, sanitisation and life span. The validation approach cannot cover all these critical parameters.

For HCP, the validation approach should only be considered for each product on a "case by case" basis depending on i) the quality of the analytical method used to identify and quantitate the HCP impurities, ii) the design and quality of the validation studies and, iii) the intended use of the product (dose, treatment, duration, etc.).

CONCLUSION

The "validation approach" appears to be an acceptable way, in most cases, to approach the question of residual host cell DNA.

This position is not as readily applicable for host cell proteins (HCP) impurities where a "case by case" review is proposed.

It is noteworthy that in any case, processes and procedures should undergo periodic critical re-evaluation to ensure that they remain capable of achieving the intended results.