The maze of extractables and leachables

– Regulatory risk assessment of β-Glucans –

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List of abbreviations

AEX ..... *Anion Exchange Chromatography*
API ..... *Active Pharmaceutical Ingredient*
BG ..... *β-Glucans*
BLA ..... *Biologics License Application*
C10 ..... *Protein A Chromatography*
CEX ..... *Cation Exchange Chromatography*
CMC ..... *Chemistry, Manufacturing & Control*
CTD ..... *Common Technical Document*
C_x *Polishing Steps*
DP ..... *Drug Product*
DS ..... *Drug Substance*
DSP ..... *Downstream Process*
E&L ..... *Extractables and Leachables*
EMA ..... *European Medicines Agency*
ERT ..... *European Registered Toxicologist*
FDA ..... *Food And Drug Administration*
GMP ..... *Good Manufacturing Practice*
HCP ..... *Host Cell Protein*
I10 ..... *Depth Filtration*
I20 ..... *Virus removal filtration*
I40 ..... *DS Formulation*
ICH *International Council for Harmonization of Technical Requirements for Pharrmaceuticals for Human Use*
IEX ..... *Ion Exchange Chromatography*
IMPD ..... *Investigational Medicinal Product Dossier*
IND ..... *Investigational New Drug Application*
IPC ..... *In-Process Control*
$_{IV}$ *Intraveneous*
LAL ..... *Limulus amebocyte lysate*
MAA ..... *Marketing Authorization Application*
mAbs ..... *Monoclonal Antibodies*
MDD ..... *Maximum Daily Dose*
MHRA ..... *Medicines and Healthcare Products Regulatory Agency*
PAS ..... *Prior Approval Supplement*
PDA ..... *Parenteral Drug Association*
PDE ..... *Permitted Daily Exposure*
PPQ ..... *Process Performance Qualification*
PRI ..... *proces-related impurities*
PV ..... *Process Validation*
QRM ..... *Quality Risk Management*
TFF ..... *Tangential Flow Filtration*
UF/DF ..... *Ultra- and Diafiltration*
USP ..... *Upstream Process*
V10 ..... *Virus Inactivation*
WHO ..... *World Health Organization*

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1. Introduction

β-Glucans (BG) are defined as a large class of natural polysaccharides composed of D-glucose monomers linked through β-glycosidic bonds. Per definition, this also includes β-1,4-Glucans (cellulose), however, the term is mostly reserved for β-1,3 and mixed-linked β-(1,3-1,4) Glucans. BG are manifold distributed in nature and can be found in several organism such as yeast, fungi, bacteria, seaweed and cereals. Their properties encompass storage, structural and protective roles [1] [2] [3] [4]. The molecular structure and weight of BG can vary depending on their source. In general, BG are unbranched polysaccharides with β-1,3- and β-1,4-linked glucose units. β-1,3-BG are often linked with additional β-1,6-linked branches [5] (Figure 1).

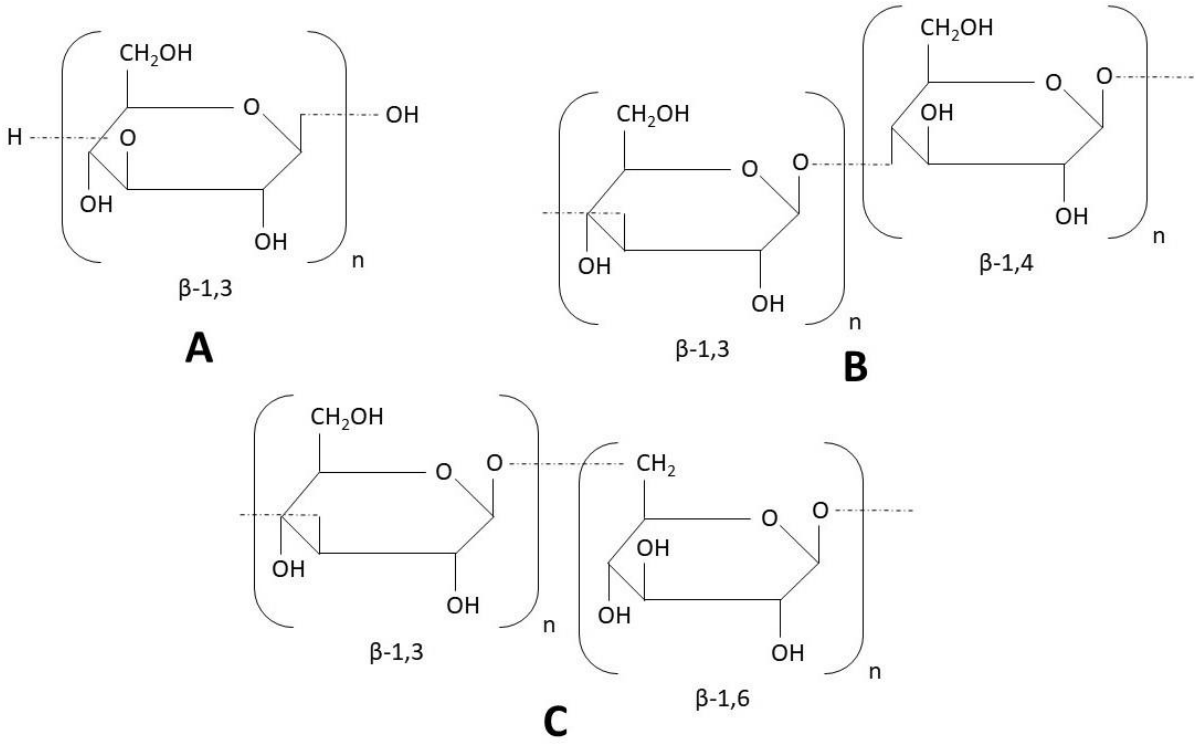


Figure 1 Molecular structure of varies BG. A: β-1,3 linked BG. B: mixed linked β-(1,3-1,4) BG. C: β-1,3 linked BG with additional β-1,6 linked branches.

Since those excellent molecular and structural features of BG determine their physical properties of these polysaccharide (technological aspects such as water solubility and their affinity towards BG interacting proteins), recent research of BGs encompassed, among others, the fields of functional, bioactive and neutraceutical ingredients [4].

In biopharmaceutical industry, bacterial endotoxin testing is part of the general microbiological analytical testing strategy. The Limulus amebocyte lysate (LAL) assay is the

compendial method for testing of bacterial endotoxins (USP <85> and Ph.Eur. 2.6.14). BG are known to cause test interference (despite use of BG blockers). This may cause worst-case situations in which raw materials or In-Process control (IPC) samples (e.g., harvest) could not be tested for bacterial endotoxins [6]. For the endotoxin content, that may have non-endotoxin pro-inflammatory impurities, such as peptidoglycan or BG, the use of a monocyte activation test or other method should be considered [7].

In recent literature [8] [9], β-1,3-BG have been found to have potential immunogenicity risk in biopharmaceutical products. β-1,3-BG are recognized as pharmaceutical contaminants, which may be introduced, inter alia, via upstream and downstream process (USP and DSP, respectively), raw materials and buffers. Consequently, β-1,3-BG (and BG in general) can be assigned to the group of process-related impurities (PRI) [10].

A typical biopharmaceutical process for monoclonal antibodies (mAbs) consists of an USP and corresponding DSP steps. In general, the USP consists of a thaw of respective cells (vial thaw), sub cultivation steps and further cultivations in respective seed bioreactors until the desired viable cell concentration is met to inoculate the production bioreactor, where the production of the desired active pharmaceutical ingredient (API) takes place. The harvest step determines the final step in USP including harvest filtration (clarified harvest). An exemplary USP flow-diagram is depicted in Figure 2 [11].

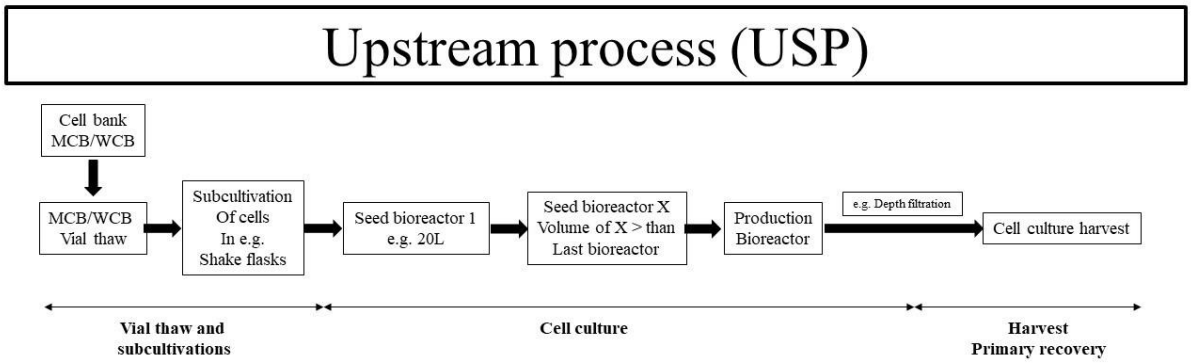
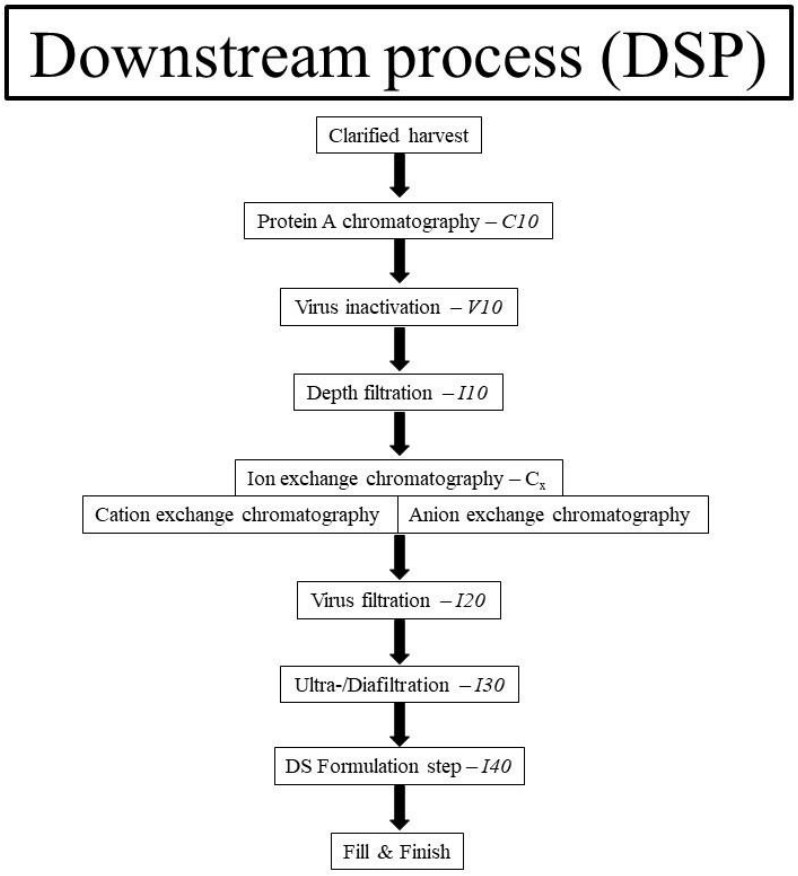


Figure 2 An exemplary Upstream Process flow-diagram. Vial thaw and subcultivations consists of the resepective vial thaw of the MCB/WCB and subcultivation of cells in shake flasks. Cell culture encompasses all seed bioreactor steps and the production bioreactor step followed by harvest of the broth. MCB: Master cell bank; WCB: Working cell bank.

In general, with the transfer of the harvest to the DSP site, the purification takes place. In difference to the USP, where the overall function can be assigned to the production of the desired API, the overall function of the DSP can be assigned to the purification of the API to meet the respective quality attributes. In general, a typical DSP starts with a Protein A (or an

equivalent step, depending on the used column, e.g., Protein L) chromatography (C10, Figure 3) to capture the desired API from the clarified bulk harvest and reduce impurities (both, product-related impurities and PRIs). Subsequently, a virus inactivation step (V10, Figure 3), to eliminate acid intolerant viruses, follows. Afterwards a depth filtration (I10, Figure 3) takes place. The function of I10 encompasses, inter alia, the removal of precipitates and reduction of host cell protein (HCP) and DNA. Polishing steps (C_x, e.g., C20, C30 etc.) – cation and/or anion exchange chromatography (CEX and AEX, respectively) – are performed after I10 (Figure 3). The function of those steps includes, inter alia, reduction of HCP and DNA, possible further virus removal (if tested) and leached Protein A (or an equivalent leachable, e.g., Protein L). To remove further virus particles, a virus removal filtration (I20, Figure 3), is carried out thereafter. The next step is an ultra- and diafiltration (UF/DF; I30, Figure 3) to exchange the matrix against the final drug substance (DS) buffer matrix and to further adjust the protein concentration. Further secondary functions (such as reduction of impurities) are possible. The final step is claimed as the DS formulation step (I40, Figure 3), where the final DS excipient composition and protein concentration is adjusted and final bioburden reduction filtration steps are performed, following dispensing in bags, and freezing. According to fill- and finish-principles, the DS is in the following aseptically filled, resulting in the respective sterile drug product (DP). Often, only aseptic filling is performed between DS and DP in mAb production, without further adjustments. An exemplary DSP flow-diagram is depicted in Figure 3 [11].



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PRIs such as BG are increasingly coming into focus of authorities. Recent experiences (business internal information, confidential) with authorities such as Food and Drug Administration (FDA) or European Medicines Agency (EMA) applications triggered questions on BG such as information on leachable studies and/or summaries of the risk evaluation on BG, in particular β-1,3-BG, which should be considered as potential leachable from the use of cellulose filters in the manufacturing process. Those experiences also call for adequate control strategies of this impurity.

The recent authority requests are only the beginning of a growing trend in industry and among regulatory authorities to detect and quantify BG, especially β-1,3-BG, and understand their safe levels. In recent regulatory associated literature, only one publication with a specification limit of 10 ng per mL mAb product (corresponds to 500 ng total dose) was identified and this has been accepted by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) [12].

As a consequence of the lacking regulatory associated literature in the context of BG there are currently no clear specifications available for acceptable levels of BG in general. Furthermore, regulatory guidelines do not offer a clear direction, yet, but resemble more a maze. BG may be introduced as leachable (inter alia leaching of BG from cellulose-based filters) but also as impurities (e.g., as an impurity of raw materials). Due to the manifold introduction of BG into the biopharmaceutical process, International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q3 quality guidelines may be (partly) applicable and should therefore be considered for assessment of BG. However, neither ICH Q3A Impurities in new DS [13] nor ICH Q3B Impurities in new DP [14] are applicable for biological/biotechnological products since those are excluded from the scope of the two guidelines. Currently, a new ICH Quality guideline (ICH Q3E Impurity: Assessment and Control of Extractables and Leachables for Pharmaceuticals and Biologics) is in preparation to face the regulatory guideline gap of not having an ICH Q3 Impurities quality guideline in place for biopharmaceutical products. According to the ICH Q3E concept paper [15] no internationally harmonized guidance on Extractables and Leachables (E&L) assessment and control exists (including reporting thresholds, safety assessments and alignments to the principles of science-based, risk-based and quality-by-design approaches). The ICH Q3E concept paper defines an extractable as:

"[…] any chemical entities that will extract from components of a manufacturing or packaging system into a solvent under forced conditions" – ICH Q3E concept paper [15]

The ICH Q3E concept paper further defines a leachable as:

"[…] that can migrate via contact with manufacturing systems, container-closure systems, and drug delivery device components" – ICH Q3E concept paper [15]

According to those definitions, it can be anticipated that BG are leachables in the context of the upcoming ICH Q3E and are therefore subject of the current regulatory guideline gap of not having an Impurities quality guideline in place for biopharmaceutical products. The lack of aligned E&L guidance framework (and all associated issues such as safety assessments in the context of risk-based approaches and respective control options as described in [15]) makes it indispensable to use general quality risk management approaches according to ICH Q9 Quality Risk Management [16] for the risk assessment of such PRIs/leachables like BG.

Furthermore, for impurities, Good Manufacturing Practice (GMP) principles are in place according to ICH Q7 Good Manufacturing Practice [17]. It is stated in ICH Q7, that for production and IPCs an adequate contamination control should be in place. Laboratory controls should also be in place especially for testing of intermediates and API. For biotechnology considerations, ICH Q7 refers to ICH Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products [18] where it is stated that the characterization, which also includes impurities, of a biotechnological product is necessary to establish relevant specifications. According to ICH Q6B, impurities may be either process (PRI) or product-related and should be adequately controlled. However, it is also stated, that for certain impurities, testing may not be necessary and may not needed to be included in the respective specifications if efficient control or removal to acceptable levels is demonstrated by suitable studies. This concept is barely not published and therefore unknown for BG. Even though no regulatory guidance for leachables/PRI such as BG are available now, some pharmacopeia chapters have been implemented recently (in particular USP <665> and <1665>) or were already published several years ago (USP <1663> and <1664>). However, under the consideration of the current gap of regulatory framework, uncertainties for industry and regulators exists due to lack of clarity meeting regulatory expectations. Consequently, lack of harmonized guidance complicates regulatory assessment. This complication creates potential delays in the approval of regulatory applications and ultimately delay in the accessibility of medicines to patients [15].

In FDA Guidance for Industry: Immunogenicity assessment for therapeutic protein products, BG are listed as an impurity with adjuvant activity from microbial or host-cell related sources. In that document, it is recommended to reduce the respective amount and using assays with appropriate sensitivity [9]. The World Health Organization (WHO) Guidelines for the production and quality control of mAB and related products intended for medicinal use clearly states out, that (on DS level) testing for BG should also be considered, particularly if the host cell is known to generate BG or if cellulose filters are used [7].

According to ICH Q11 Development and Manufacture of Drug Substances, the respective USP and DSP steps should, inter alia, be designed to reduce impurities such as BG [19]. Since regulatory authorities will assess whether the controls of DS and their respective manufacturing process can be considered adequate, the DS manufacturing process should be

sufficiently described in the application to understand how impurities entry the process, which changes they undergo, how they will be removed and why the proposed control strategy is suitable for the remaining impurities [19]. The scope of an application differs depending on the respective clinical phase (Phase I, II and III respectively) and entry into market through a marketing application. Since drug development and manufacturing processes grow over time, the knowledge of impurities is rather low beginning of Phase I and will extend to higher knowledges until marketing applications. Further knowledge of impurities will be gained post-authorization. Consequently, the information on impurities will only be limited through a respective Investigational New Drug Application (IND; [20] [21]) and Investigational Medicinal Product Dossier (IMPD; [22]) and will expand through the different clinical phases [23] [24]. Deep process understanding will be gained through process validation (PV; [25] [26]) procedures resulting into respective biologics license application (USA – BLA) and marketing authorization application (EU – MAA). Both, BLA and MAA, follows the Common Technical Document (CTD) structure [27]. For impurities, such as BG, the ICH M4Q Common Technical Document for the Registration of Pharmaceuticals for Human Use: Quality [28] lays down the respective structure for implementation into the quality module of the respective marketing authorization.

2. Objectives

Since there exists an uncertain regulatory environment concerning leachables and/or impurities such as BG, there is a special regulatory need to assess BG as potential leachable and/or impurity in a risk-based approach to walk through this maze and built a clearer path. The lack of aligned regulatory guidance framework makes it indispensable to use general quality risk management approaches according to ICH Q9. Consequently, this master thesis will be built up according to risk management principles layed down in ICH Q9.

The first aim of this master thesis will therefore encompass the development of a control-strategy of BG that will be (most likely) accepted by the competent authorities at market submission stage. This will include a risk-assessment according to ICH Q9 as well as a respective testing strategy of BG.

Subsequently, as second aim, the risk assessment will be discussed, interpreted and embedded in the regulatory framework. In this context, it will be discussed and interpreted, if a respective testing strategy is necessary at different clinical phases (Phase I, II and III, respectively) and if so, how it should look like. This will also be discussed for the market stage.

The third aim will be focused on the respective regulatory dossiers. It will be discussed and interpreted, if data concerning BG as leachable/impurity need to be included in regulatory submission dossiers. A regulatory strategy will be discussed, how this data should look like in the respective dossiers and which dossier section of the CTD granularity is involved. Also cross-links between different dossier sections will be discussed. This discussion will include clinical dossiers (Phase I, II and III, respectively) as well as marketing application dossiers. It will be focused on USA and EU. Furthermore, it will be discussed if changes in the commercial production for market supply will have an impact on the information of BG in dossiers. Potential regulatory changes (EU: variation; USA: changes) will be assessed, and if submissions of regulatory changes will be necessary, it will be discussed, which form of change needs to be submitted.

The fourth aim will take a look in the closer future and will analyze and discuss if the regulatory gap of extractables and leachables can be fully covered with regulatory risk assessments. This will also be discussed in the context of the implementation of ICH Q3E.

Overall, the outcomes of this master thesis will give deep industry-relevant regulatory and technically knowledge in the context of leachables/impurities, namely BG. It will give a strategy to support and facilitate regulatory applications in the biopharmaceutical industry through suggestions for test- and control-strategies as well as a clinical-phase dependent recommendations and discussions about the handling of new leachables/impurities data of BG in regulatory dossiers (IND/IMPD/BLA/MAA) and post-marketing if regulatory changes occur.

3. Materials and Methods

The following section will briefly describe all Materials and Methods used for this master thesis. The author would like to remark, that the risk assessment was conducted according to ICH Q9, therefore the basic framework of ICH Q9 is briefly described in this section.

3.1 Risk assessment according to ICH Q9

The risk-assessment for BG was conducted according to a typical quality risk management process described in ICH Q9 (Figure 4).

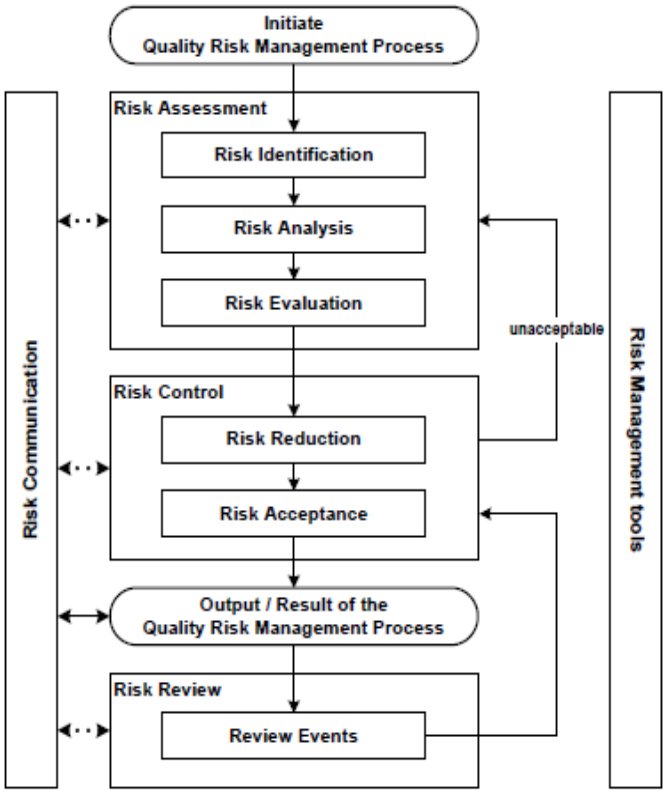

Figure 4 A typical quality risk-assessment process according to ICH Q9. The same scheme was used as described in ICH Q9.

3.1.1 Initiate Quality Risk Management Process

For initiation and planning of a quality risk management (QRM) process the following steps were included:

- Definition of problem and risk question
- Literature research for background information and data collection on the potential impact relevant to the risk assessment

Further deliverables such as timelines, appropriate levels of decision making and forming of an interdisciplinary team were also part of this process, however this will not be described in this thesis.

3.1.2 Risk assessment

The actual risk assessment encompassed three steps, which were the identification of risk, the analysis of which and a respective evaluation of the identified and analyzed risk.

Risk Identification

Information for risk identification included literature research and data/information gained through actual analysis (business internal information, confidential). This information provided the basis for further steps in the QRM process.

Risk Analysis

This step encompassed the qualitative process of linking the likelihood of occurrence and severity of harms. Therefore, the risk analysis formed the estimation of the risk associated with the identified hazard.

Risk Evaluation

This step evaluated the outcomes from the risk analysis and brought the identified and analyzed risk in the context of the risk criteria given.

3.1.3 Risk Control

The risk control was fundamental for the risk assessment and defined the control of the identified risks after risk evaluation. There were two different approaches of risk control, which were risk reduction and risk acceptance. In general, the purpose of the risk control was to reduce the risk to an acceptable level.

Risk Reduction

This part of risk control was especially important when a risk exceeded a specified (acceptable) risk. Therefore, risk reduction focused on process for mitigation of that risk to acceptable levels.

Risk Acceptance

Risk acceptance could either be the next step of a risk reduction process (when a risk is under control and does not exceed a specified risk level) or a stand-alone process after risk evaluation. In general, risk acceptance was a decision to accept the risk. This could either be a formal decision to accept the residual risk (which could be the case after a risk reduction strategy) or a passive decision in which residual risks were not specified. Therefore, as part of the risk acceptance process, it was important to know the respective risk sources and to qualify and/or quantify those sources (according to the risk assessment process described above).

3.1.4 Output / Result of the Quality Risk Management Process

For the sake of this risk assessment, the output was a qualitative description of a range of risk and was defined in as much detail as possible. The output of the QRM process was appropriately documented and communicated to all relevant stakeholders and a risk review process was implemented to have a mechanism in place for reviewing new knowledge.

3.1.5 Risk Communication

This step included the sharing of information about risk between decision makers and other parties. Risk communication was part along the QRM process and the output/result of the QRM process was appropriately communicated and documented (Figure 4; ICHQ9). Since this step is extremely dynamic and was included along the whole risk assessment process, this step will not be described in this master thesis. However, the author wants to emphasize, that risk communication was part of the whole QRM process.

3.1.6 Risk Review

In general, QRM processes are dynamic processes since only the status quo risk is assessed. However, it is always possible that new knowledge and experiences will be gained. Therefore, the output/results of the QRM process also implemented a mechanism to review or monitor events. This should be assessed in the risk review step. Since the risk review looks into the future, this step will not be discussed in this master thesis. However, for the whole risk assessment, a risk review process was implemented and includes reconsideration of risk acceptance decisions, if needed.

3.2 Literature research

For literature research, the research websites PubMed® (https://pubmed.ncbi.nlm.nih.gov/) was used as primary research engine and Google Scholar (https://scholar.google.de/) was used as secondary research engine, if not all relevant research was published in the primary research engine.

To conduct the literature research Boolean operator "AND" was used. The following combination of words were used for literature research in PubMed® (Table 1):

Table 1 Literature research with PubMed®. The Boolean operator "AND" was used. It is shown which word combination were used and how many numbers of hits were found. After reading of all abstracts, the quality of hits was categorized in "usable" and "not usable". To be eligible as "usable" in the context of this master thesis, the respective publication in question should be either a relevant publication with published data on the fate of BG or a review with a summary of relevant publications.

Combination	***Number of hits***	***Quality of hits***
((beta glucane[Title/Abstract]) AND (contamination) AND (downstream)	3	3 publications usable
((beta glucane) AND (downstream)) AND (biopharmaceutical)	83	3 publications usable
((beta glucane) AND (impurity)) AND (downstream)	3	2 publications usable
(beta glucane[Title/Abstract]) AND (downstream[Title/Abstract])	63	4 publications usable
((beta glucane) AND (chromatography)) AND (process)	126	4 publications usable
((beta gluicane) AND (membrane)) AND (downstream)	38	3 publications usable
((beta glucan) AND (clearance)) AND (downstream)	9	2 publications usable
Overall publications after deletion of doubling publications: 5 publications		

The following combination of words were used for literature research in Google Scholar (Table 2):

Table 2 Literature research with Google Scholar. The Boolean operator "AND" was used. It is shown which word combination were used and how many additional numbers of hits were found in addition to the literature research described in Table 1. After reading the abstracts of the additional publications, the quality of hits was similar categorized as described in Table 1.

Combination	***Additional publications***
((beta glucane[Title/Abstract]) AND (contamination) AND (downstream)	1 additional publications

((beta glucane) AND (downstream)) AND (biopharmaceutical)	1 additional publications
((beta glucane) AND (impurity)) AND (downstream)	No additional publication
((beta glucane) AND (chromatography)) AND (process)	No additional publications
((beta gluicane) AND (membrane)) AND (downstream)	No additional publications
((beta glucan) AND (clearance)) AND (downstream)	No additional publications
2 additional publications were found in Google Scholar	

For background information and data on potential hazard, harm or human health impact relevant to the risk assessment, the literature research revealed 7 publications. The list of publications is depicted in Annex I.

3.3 PDE assessment

A permitted daily exposure (PDE) value assessment for BG was commissioned from Rentschler Biopharma SE to an external European registered toxicologist (ERT) after literature research identified the necessity for calculating a respective PDE for BG. The author would like to remark, that this document is confidential and will therefore not be part of the bibliography section. Briefly, a $PDE_{Intraveneous\ (IV)}$ of 3 µg/day was derived from a subacute toxicity study. Since the PDE_{IV} corresponds the systemic PDE, no further safety factor needs to be applied.

$$PDE_{IV} = 3\ \frac{\mu g}{day} = PDE_{systemic}$$

3.4 Calculations

To assess the criticality of BG contamination in DS of analyzed mAb samples, the following calculations were used in the results section (Table 3):

Table 3 Assessment of criticality of BG contamination in DS of all analyzed mAb samples produced at Rentschler Biopharma SE. The steps (A) and (B) are business internal information and therefore confidential. To use worst-case approaches, the BG levels found in the analyzed mAb samples were further looked at in the context of the maximum daily dose (MDD) to cover maximum potential contamination of BG (C). Since the expected BG amount per MDD is compared to the PDE, it can be concluded with this formula if there exists toxicological concerns or not (F).

Steps	Levels mAb 1-X	Remarks

(A) BG levels in DS in pg/mg	Criticality assessment starts with analytical determined BG levels in DS – crucial for knowledge of actual BG contamination on DS level.	Business internal information. Confidential.[1]
(B) Maximum Daily Dose (MDD) in mg	For worst case estimation, MDD is used to cover maximum potential contamination of BG.	
(C) BG per day, when MDD is applied in pg/day ((A)x(B))	Multiplication of (A) and (B) to cover maximum potential contamination of BG.	Worst case approach.[1]
(D) BG per day, when MDD is applied in µg/day = expected BG amount per MDD ((C)/10^6)	Mathematical conversion.	
(E) PDE_{IV} 3 µg BG per day	3	PDE assessment.
(F) Comparison of expected BG amount per MDD and PDE ((D)/(E)) = Factor (F)	Division of (D) and (E) to compare the maximum expected BG amount with the PDE. If Factor (F) < 1 – no toxicological concern. If Factor (F) > 1 – toxicological concern.	Calculation of factor.

[1] The author would like to remark, that this information is confidential and will therefore not be given in this master thesis.

4. Results

The result section encompasses the risk assessment according to ICH Q9, which is the first aim of this master thesis (as described in section 2). For that, the risk assessment is embedded into the ICH Q9 framework (as described in section 3.1). After the results section, which includes the actual risk assessment, the second aim as well as the third and fourth aim of this master thesis (as described in section 2) will be discussed and interpreted in the discussion section (see section 5).

4.1 Initiate Quality Risk Management Process

The master thesis and the respective risk assessment according to ICH Q9 for BG was triggered based on recent experiences (business internal information, confidential) with authorities such as FDA or EMA for mAb marketing applications. Questions on BG such as information on leachable studies and/or summaries of the risk evaluation on BG, in particular β-1,3-BG, which should be considered as potential leachable from the use of cellulose filters in the manufacturing process were requested. Those experiences also call for adequate control strategies of this impurity. Consequently, the following risk question was derived from those experiences:

"Does the leaching of BG contribute to a safety problem for the product? If yes, what actions need to be taken to mitigate this risk?"

This question was further expanded to all possible entry option of BG in form of impurities. Consequently, the risk assessment was not only carried out for the leaching of BG (from the use of cellulose filters) but also for all possible impurity entry options of BG. For this specific risk question, the whole manufacturing process, including assessment of raw materials, needed to be assessed. To initiate the QRM process, literature research for background information was carried out. The research was conducted as described in section 3.2.

4.2 Risk Assessment

As first part of the respective risk assessment of BG, the risk identification (section 4.2.1) was conducted, which included literature research as well as data/information gained through actual analysis of mAb samples (section 3.2 and 3.4). At Rentschler Biopharma SE three different mAbs were analyzed in total (two mAbs with one lot each and one mAb with three

lots; the three different mAbs were named mAb 5, mAb 6 and mAb 7, respectively). The author would like to remark that raw data of actual analysis are confidential business internal information and will therefore not be depicted in this master thesis. This information will provide the basis for the subsequent step, which was the actual risk analysis (section 4.2.2), where the qualitative process of linking the likelihood of occurrence and severity of harms was analyzed. As last step, the risk was evaluated (section 4.2.3) and will bring the identified and analyzed risk in the context of the risk criteria given.

4.2.1 Risk Identification

During biopharmaceutical production of mAbs, there could be many sources of BG contamination in both USP and DSP, respectively [29]. Since contamination with BG can be manifold, literature research was conducted (section 3.2) to first gain more knowledge concerning possible entry and depletion steps of BG. For the sake of readability in accordance with ICH Q9 principles, every single step, starting with harvest, was examined more closely. Further information is depicted afterwards.

4.2.1.1 Harvest

BG impurities may be introduced in USP through cell culture feed media [30] but also by using cellulose based filter during harvest [31]. Concentration of BG in harvest (clarified) can reach up to 150.000 pg per mL [30] [31] [32]. Table 4 shows the BG concentration at harvest found in literature search.

Table 4 BG concentration at harvest found in the literature. It is indicated in the footnotes which publication is used for mAb 1, mAb 2, mAb 3 and mAb 4. BG concentration are depicted in [pg/ml] and [pg/mg] because some publications calculate the respective BG concentration in either [pg/mL] or [pg/mg] or both.

mAb		BG [pg/mL]	BG [pg/mg]
mAb 1 [1]		73 928	32 143
mAb 2 [1]		152 059	56 318
mAb 3 [2]	Lot 1	142 863	N/A
	Lot 2	139 411	N/A
	Lot 3	98 460	N/A
mAb 4 [3]		25402.61	N/A

[1] Wang et al. [30]

[2] Jiang et al. [32]

[3] Vigor et al. [31]

A contamination of BG during USP, based on the data presented in Table 4, is probable. This finding is also strengthened by one mAb (mAb 5) produced at Rentschler Biopharma SE, however lower BG contamination (between $\frac{1}{3}$ to $\frac{1}{5}$ lower contamination compared to the literature; business internal information, confidential) were found. With high probability, cell culture feed media and/or the use of cellulose filter during harvest could contribute to a BG contamination.

4.2.1.2 C10 - Protein A chromatography

A high clearance of BG (clearance factor up to 99.99% post-Protein-A chromatography step, equal to a 4 $\log_{10}$ reduction; [30] [32]) was observed in the literature. For one mAb (mAb 5) produced at Rentschler Biopharma SE, also approximately 3 to 4 $\log_{10}$ reduction was achieved. For another mAb (mAb 6) produced at Rentschler Biopharma SE, only post-Protein-A data were available, however this data was also in the same range as the other mAb (business internal information, confidential). Similarly, Vigor et al. [31] also achieved approximately a 3 to 4 $\log_{10}$ reduction. Furthermore, Kluters et al. [29] found similar BG concentrations after Protein A chromatography. However, due to lacking data of harvest results, no clearance factor could be determined. Table 5 summarizes the BG concentration after Protein A chromatography found in the literature.

Table 5 BG concentration after Protein A chromatography found in the literature. It is indicated in the footnotes which publication is used for mAb 1, mAb 2, mAb 3 and mAb 4. BG concentration are depicted in [pg/ml] and [pg/mg] because some publications calculate the respective BG concentration in either [pg/mL] or [pg/mg] or both.

mAb		BG [pg/mL]	BG [pg/mg]
mAb 1 [1]		38.2	4.3
mAb 2 [1]		79.6	4.6
mAb 3 [2]	Lot 1	172	N/A
	Lot 2	73	N/A
	Lot 3	109	N/A
mAb 4 [3]		7.17	N/A

[1] Wang et al. [30]

[2] Jiang et al. [32]

[3] Vigor et al. [31]

It is hypothesized [32] that the specificity of the Protein A chromatography on binding mAbs is responsible for its efficacy in clearing the BG impurity. Based on the available literature [29] [31] [30] [32], it is probable that BG do not appear to bind to any of the modes of chromatography or associate in significant levels with mAbs. As the physical properties of BG appear to not be charged or significantly hydrophobic (as described in section 1), BG tend to flow through most modes in chromatography. The high clearance properties of the Protein A chromatography are not only consistent throughout the literature but also been shown internally (mAb 5 and mAb 6, business internal information, confidential).

4.2.1.3 V10 – Virus inactivation

In general, a virus inactivation step follows the C10 step (Protein A chromatography) for purification of mAbs. Elimination of acid intolerant viruses define the function of this step [11]. This is accomplished through achieving a defined target pH. It can be assumed that no BG contamination or depletion will occur through this step. Wang et al. [30] measured the BG clearance from harvest to neutralized viral inactivated product (which means BG was measured after harvest and after Protein A chromatography/Virus inactivation step; data as shown in Table 5). The measured clearance was comparable to the BG clearance after Protein A chromatography only.

4.2.1.4 I10 – Depth filtration

Cellulose is commonly used for depth filter. As described in the Parenteral Drug Association (PDA) technical report no. 45 [33], leachable levels of contaminants (e.g. BG) should be characterized. Cellulose may leach significant quantities of BG, which defines cellulose derived depth filter as a potential source of BG contamination. Filtration tests showed that BG can leach from cellulose derived depth filter [34] [35] [36]. It was also shown that the use of synthetic depth filter avoids leaching of BG, since those filters would not be derived from cellulose or cellulose derived material [35] [36] [37]. Furthermore, in recent literature, it was investigated if a pre-use flush strategy could reduce BG contamination. Gefroh et al. [38] as well as Holstein et al. [37] showed that a respective pre-use flush strategy could reduce BG contamination. Holstein et al. [37] also investigated, that greater BG leaching may occur at

higher pH levels. Kluters et al. [29] found a potential inverse dependency between depth filtration load volume and BG content in filtrate. However, Holstein et al. [37] observed no significant changes when applying different filter loadings. Not all vendors investigate the BG leaching for their cellulose derived filters. It was found that for some depth filter (according to validation guides of vendors; business internal information, confidential) the BG leaching was investigated by the vendors themselves. In general, the vendors explain in their validation guides, that the customers must flush filters before exposure to their products. The product pool after cellulose derived depth filtration as a potential source of BG contamination was also investigated for two mAbs (mAb 5 and mAb 6) produced at Rentschler Biopharma SE. It was found that the BG concentration increases in both mAbs (mAb 5 and mAb 6, respectively) as in comparison to post-Protein-A-chromatography (business internal information, confidential). Therefore, it can be concluded that cellulose derived depth filter may introduce BG into the process.

4.2.1.5 C_x – Polishing steps

Independent of the respective form of ion exchange chromatography (IEX; CEX or AEX, respectively) the IEX can be constituted in flow-through or in bind-and-elute mode. BG clearance properties were only found in bind-and-elute mode throughout the recent literature. Kluters et al. [29] as well as Holstein et al. [37] investigated IEX in flow-through mode and found that IEX flow-through mode do not remove BG sufficiently. Furthermore, Kluters et al. [29] found that IEX in bind-and-elute-mode may probably be an efficient clearance step for BG. Table 6 describes the BG contamination in different mAbs from different publications. It is indicated in the footnotes whether flow-through or bind-and-elute mode was used.

Table 6 BG concentration after polishing steps found in the literature. It is indicated in the footnotes which publication is used for mAb 1, mAb 2, mAb 3 and mAb 4. BG concentration are depicted in [pg/ml] and [pg/mg] because some publications calculate the respective BG concentration in either [pg/mL] or [pg/mg] or both. It is further indicated in the footnotes which ion exchange chromatography (cation or anion, respectively) and which mode was used.

mAb		BG [pg/mL]		BG [pg/mg]
mAb 1 [1]		70.7[4]		8.6[4]
mAb 2 [1]		44.6[4]		5.0[4]
mAb 3 [2]	Lot 1	8[5]	<6.25[4]	N/A
	Lot 2	12[5]	7[4]	N/A

	Lot 3	<12.5[5]	<6.25[4]	N/A
mAb 4 [3]		22.92	6.13	N/A

[1] Wang et al., [30]

[2] Jiang et al., [32]

[3] Vigor et al., [31]

[4] Flow-through mode AEX chromatography.

[5] Bind-and-elute mode CEX chromatography.

Wang et al. [30] investigated two mAbs in flow-through AEX chromatography mode. This mode could not sufficiently remove BG (no significant change when compared to BG concentration after Protein-A-chromatography step; see Table 5). Consequently, no $\log_{10}$ reduction (-0.3 to -0.0, respectively; [30]) was observed. Jiang et al. [32] used both, a bind-and-elute CEX as well as a flow-through AEX. For bind-and-elute CEX chromatography a clearance factor of $\log_{10}$ 1 was observed. No further reduction was observed for flow-through AEX chromatography, however the load concentration BG after CEX was very low, therefore no assessment of flow-through AEX was possible. Spiking experiments confirmed the hypothesis, that bind-and-elute provide sufficient clearance whereas flow-through mode does not provide any clearance at all [32]. The data from the mAb investigated by Vigor et al. (mAb 4, Table 6, [31]) could not be assessed as a potential removal step because after the Protein-A chromatography step, the BG concentration was low (Table 5) and no further reduction could be observed. Spiking experiments were not conducted. The data from the recent literature was confirmed by the data from two mAbs (mAb 5 and mAb 6, respectively) produced at Rentschler Biopharma SE, where flow-through modes could not remove significant BG concentrations (business internal information, confidential). Bind-and-elute IEX modes were not investigated at Rentschler Biopharma SE. Similar to the Protein-A chromatography step, BG tend to flow through most modes in chromatography. Consequently, in bind-and-elute mode, the respective mAb will be captured by the resin of the column, whereas the BG will flow through the column without any significant interaction with the resin of the column.

4.2.1.6 I20 – Virus filtration

Like depth filter, virus removal filter may be constituted from cellulose derived material, therefore potential leaching of BG could occur. Wang et al. [30] investigated for mAb 1 and mAb 2 (see Table 7) the potential leaching of BG after virus removal filtration.

Table 7 BG concentration after virus filtration found in the literature. It is indicated in the footnotes which publication is used for mAb 1 and mAb 2. BG concentration are depicted in [pg/ml] and [pg/mg] because some publications calculate the respective BG concentration in either [pg/mL] or [pg/mg] or both.

mAb	BG [pg/mL]	BG [pg/mg]
mAb 1 [1]	80.7	10.3
mAb 2 [1]	626	116.0

[1] Wang et al., [30]

Wang et al. [30] found for mAb 2 increased BG levels after virus filtration step, however for mAb 1 the BG level range was within method variation. Contrarily, Kluters et al. [29] found no major increase in BG content after virus filtration step, however the virus filter was flushed prior to loading. Potential leaching of BG may occur, if no flush strategy prior loading of filter is performed [31] [38]. Vigor et al. [31] investigated BG levels in water flush samples and found, that the filter housing storage buffer could be the source of BG impurity. A respective filter flush strategy reduced the BG contamination. Gefroh et al. [38] found similar results and concluded that initial water and equilibration flushes could remove BG leachables. Similar to depth filter, not all vendors investigate the BG leaching for their cellulose derived filters. It was found that for some virus filter (according to validation guides of vendors; business internal information, confidential) the BG leaching was investigated by the vendors themselves. Consistent with the literature, the vendors refer to flushing strategies. No data at Rentschler Biopharma SE were obtained concerning virus filtration to confirm the literature.

4.2.1.7 I30 – Ultrafiltration and Diafiltration

Like depth filter and virus filter, UF/DF may also be constituted from cellulose derived material. Therefore, potential contamination through leaching of BG from UF/DF filter may be possible. However, also a potential clearing property of UF/DF filter is discussed in the literature. Kluters et al. [29] observed inconsistent clearing properties for two different mAbs. It is hypothesized that different clearance may be related to BG molecular species of different

molecular weight being present in UF/DF load. Similar observations were made from Gefroh et al. [38], Vigor et al. [31] and Jiang et al. [32]. Interestingly, Jiang et al. [32] further hypothesized that smaller pore size membranes of UF/DF filters (e.g., 10 kDa) could provide clearance, however such tight membranes are typically not used for mAb processes because of the lower flux rates. Wang et al. [30] investigated for mAb 1 and mAb 2 (see Table 8) the potential leaching of BG after UF/DF step.

Table 8 BG concentration after Ultra- and Diafiltration found in the literature. It is indicated in the footnotes which publication is used for mAb 1 and mAb 2. BG concentration are depicted in [pg/ml] and [pg/mg] because some publications calculate the respective BG concentration in either [pg/mL] or [pg/mg] or both.

mAb	BG [pg/mL]	BG [pg/mg]
mAb 1 [1]	517	7.8
mAb 2 [1]	3974	79.2

[1] Wang et al., [30]

For mAb 1 and mAb 2 [30] the range of BG content was within method variation, the higher BG levels in pg/mL unit were observed due to up concentration in UF/DF. Therefore, no clearing but also no contamination properties were observed for those mAbs. Similar results were observed for mAb 6 produced at Rentschler Biopharma SE (business internal information, confidential). Interestingly, for mAb 5 produced at Rentschler Biopharma SE (business internal information, confidential) the BG concentration was approximately 80 times lower than for mAb 6. For mAb 5 and mAb 6, the same UF/DF cassettes were used. This observation strengths the hypothesis that different clearance but also contamination may be related to BG molecular species of different molecular weight being present in cellulose derived UF/DF filter. Since this heterogeneous distribution is most of the times barely unknown and a variable, which cannot be easily controlled, possible clearance but also contamination properties cannot be ruled out.

4.2.1.8 I40 – DS

If not performed in I30, the I40 step generally encompasses the DS formulation step, where excipients are added, and the formulation will be finalized. According to Kluters et al. [29] final BG concentration in final DS ranged from 0.9 to 11.4 pg/mg for their investigated mAbs. Data for BG content from DS was also investigated in one mAb (mAb 7) produced at Rentschler Biopharma SE on three different lots. The BG content of the investigated lots from

the mAb 7 were in the same range as described in the literature (business internal information, confidential). Consequently, the same range was observed for mAb 7 and the mAbs investigated by Kluters et al. [29].

4.2.1.9 Overall BG process map overview for mAb 5, mAb 6 and mAb7

The fate of BG during the DSP is mapped for mAb 5, mAb 6 and mAb 7 (business internal information, confidential) in Figure 5. The author would like to remark that only the fate is depicted in Figure 5 but without actual data since this data remains confidential and will not be presented in this master thesis.

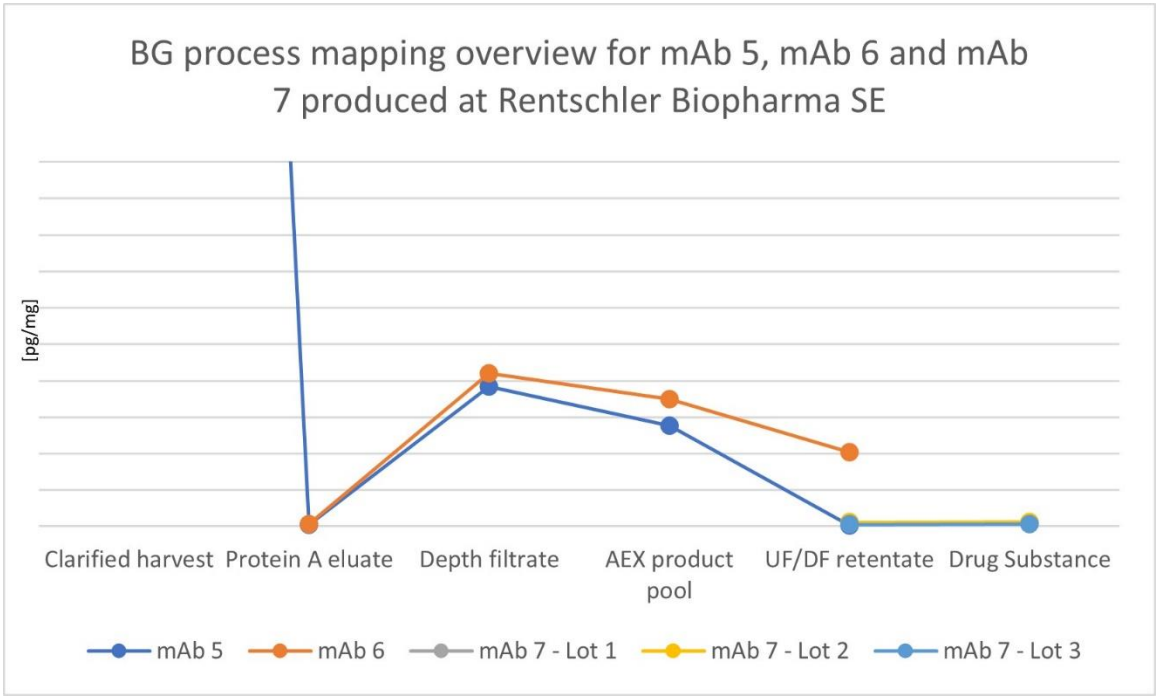

Figure 5 Fate of BG during DSP for all mAbs produced at Rentschler Biopharma SE (mAb 5, mAb 6 and mAb 7, respectively). Since the actual data of the single steps are confidential, only the fate but not the actual data is depicted in this figure. For mAb 5 and mAb 6 flow-through mode was used for AEX. For mAb 5 and mAb 6 data of Protein A chromatography, depth filtration, AEX and UF/DF was assessed. Furthermore, for mAb 5 data on clarified harvest was assessed. For mAb 7, only data on UF/DF and DS step was assessed.

Figure 5 shows, that extremely high BG concentration can occur after USP (clarified harvest mAb 5). As described in the literature, the same excellent clearing properties of the Protein A chromatography could be shown (mAb 5 and mAb 6, respectively). A contamination property of the depth filtration step, similar to the literature, could also be shown (mAb 5 and mAb 6, respectively). For mAb 5 and mAb 6, the hypothesis that flow through chromatography does not clear BG could be shown. Interestingly, contrary results for mAb 5 and mAb 6 were observed at UF/DF step. No DS data were investigated for mAb 5 and mAb

6. For mAb 7, UF/DF and DS data were collected and showed consistent low BG levels throughout the investigated lots.

4.2.1.10 Steps to be further looked at for Risk Analysis

With all information outlined in the literature and the data gathered at Rentschler Biopharma SE for mAb 5, mAb 6 and mAb 7, some assumptions can be made to take the next step in the QRM process.

First, based on the data from the literature and business internal information, it can be assumed that the last depletion step in DSP of mAbs is the last bind-and-elute IEX step C_x (both, AEX and CEX, respectively). It is important, that this step is performed in bind-and-elute and not in flow-through mode, since it was shown (both, in literature and internal, respectively), that BG tend to only flow-through the respective chromatography step. Therefore, the capture of the respective mAb of interest plays a pivotal role in depletion of BG. With a powerful reduction capacity in Protein A chromatography and a moderate clearance capacity at the last bind-and-elute IEX step, it can further be assumed that all BG introduced prior the last bind-and-elute IEX step is negligible in the context of the overall contamination of BG in DS. Consequently, a subsequent risk analysis is not necessary for all steps and sources of contamination of BG prior the last bind-and-elute IEX step.

Second, all possible entry options of BG for the overall contamination in DS needs to be taken into consideration for all steps after the last bind-and-elute IEX step. Potential contributors of BG impurities to the DS after the last bind-and-elute IEX step are shown in Figure 6.

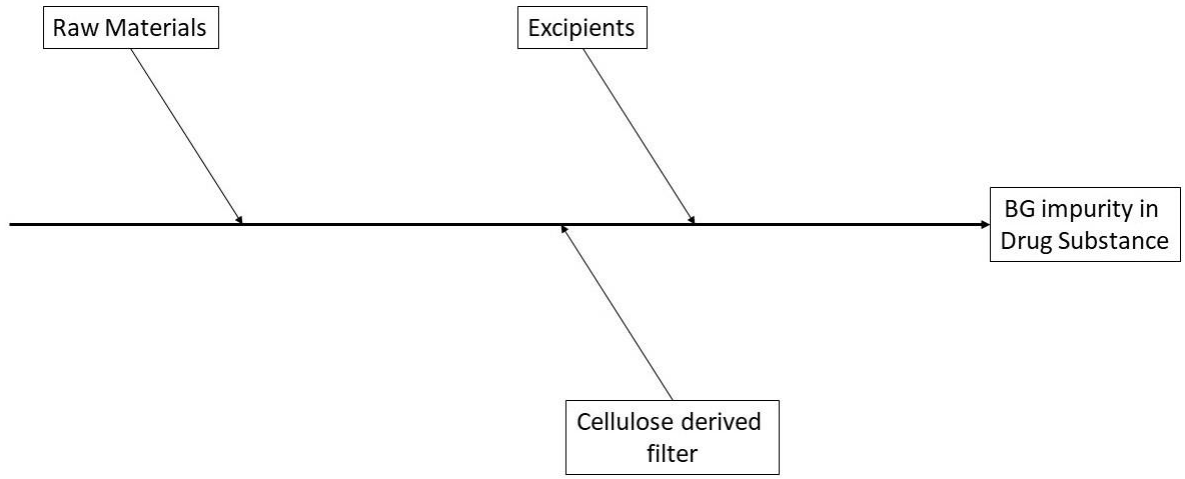

Figure 6 Potential contributors of BG impurities to the DS after last bind-and-elute IEX step.

In the next step, Risk Analysis, the potential contributors as shown in Figure 6 will be analyzed. Furthermore, calculations (for calculations see section 3.4) will be executed to identify, if the BG contamination found in diverse mAbs are critical in the context of the PDE (for PDE assessment see section 3.3). A categorization of the derived PDE into the literature and usage of those will also be discussed in this section.

4.2.2 Risk Analysis

4.2.2.1 Raw Materials and Excipients

Different to a raw material, an excipient used in the manufacturing of a biotechnological product is according to ICH Q6B [18] “an ingredient added intentionally to the DS which should not have pharmacological properties in the quantity used”. In general, raw materials are designated as excipients when they are included in the final formulation of DS.

BG can be present in several raw materials used in biomanufacturing, including but not limited to plant-derived raw materials, cotton-containing enclosures and fungi or yeast hydrolysate [10] [12] [31] [32] [6] [39] [40]. Based on the assumption, that raw materials introduced after the last bind-and-elute IEX step are important for this risk assessment, the plant-derived raw materials play a potential pivotal role in the introduction of BG in DS. In this context, the most found raw material containing a BG contamination was sucrose and sucrose-containing buffers [39] [31] [38]. Vendors reacted to that founding and offer now highly purified sucrose with significant reduction of BG contamination [41]. Furthermore, citric acid and sodium citrate was also found to have BG contamination. However, it seems that this phenomenon underlays lot-to-lot variability [38].

BG blocking agent is used in bacterial endotoxins testing if bacterial endotoxins can not be tested due to interference (see section 1). For risk analysis, it was screened for which raw materials BG blocking agent is used at Rentschler Biopharma SE (business internal information, confidential). It should be emphasized here, that the use of BG blocking agent is not proof for existence of BG in the raw material of question, however it suggests that it could be possibly contaminated. Only three DSP-relevant raw materials needed the use of BG blocking agents (business internal information, confidential). For those raw materials the vendors manufacturing processes were checked. It was found for all concerned raw materials, that they were either plant-based or cellulose filter were used during purification. However,

vendors generally do not test their raw materials for BG. This leads to a knowledge gap in the context of BG introduction into the process through raw materials, since many raw materials underly lot-to-lot variabilities. Therefore, it can be concluded that all raw materials used after the last bind-and-elute IEX step should be classified as critical in the context of BG contamination properties and therefore as a potential source to jeopardize patient safety.

4.2.2.2 Cellulose derived filter

BG can be introduced through manifold cellulose derived filter. It was also shown in recent literature, that BG contamination can be reduced through pre flushing strategies (section 4.2.1).

All cellulose derived filter used at Rentschler Biopharma SE in DSP were checked for data from vendors concerning BG leaching. Extractable data and validation guides of 101 filters from different vendors were checked (business internal information, confidential). The list encompassed tangential flow filtration (TFF) membranes, virus filter, diverse membrane filter (0.2/0.45 µM for Bioburden filtration) and depth filter. It was found, that for only a few cellulose derived filter types, the respective BG leaching was tested from the vendors. For depth filter and virus filter, filter specific BG leaching properties were assessed by the vendors (business internal information, confidential). However, for all other filter types, no data were found in the respective extractable data sheets and validation guides. This must be considered rather critical since filter types different to depth and virus filter are used post last bind-and-elute IEX step. Bioburden filtration steps, often performed inline, as well as TFF membranes provide incalculable risk in the context of BG leaching from those filter types. Furthermore, virus filtration is often downstream performed from the last bind-and-elute IEX step. Consequently, it can be concluded that all cellulose derived filter used after the last bind-and-elute IEX step should be classified as critical in the context of BG contamination properties and therefore as a potential source to jeopardize patient safety.

4.2.2.3 Calculations to assess criticality of BG contamination in Drug Substance

The risk analysis for raw materials and excipients as well as cellulose derived filter resulted into critical classification after the last bind-and-elute IEX step. However, based on the initial risk question *“Does the leaching of BG contribute to a safety problem for the product? If yes, what actions need to be taken to mitigate this risk?”* (see section 4.1), the overall contamination of BG in DS should also be analyzed. Only on that basis, a respective control

strategy can be derived (for control strategy approach, see section 5.1). In recent literature, only one acceptable range of BG in DS was published [12]. Barton et al. [12] derived an BG upper limit of 500 ng per dose. This dose was also accepted from the respective authority (MHRA). However, the upper limit needs always to be considered in respect of the target population and an appropriate safety risk assessment should always be examined. A PDE assessment for BG was commissioned from Rentschler Biopharma SE to an external ERT (see section 3.3). A PDE_{IV} of 3 µg BG per day was assessed. This PDE was used to assess the criticality of BG contamination, whether the contamination of BG in DS needs to be evaluated critical or not. For this approach, the BG concentration of mAb 7 (BG concentration in DS of three lots of mAb 7) and mAb 6 as well as mAb 5 (BG concentration after UF/DF step of mAb 6 and mAb 5) were used. The BG concentration after UF/DF step of mAb 5 and mAb 6 was used since no DS data were available for those two mAbs. It was hypothesized that after UF/DF step, no significant contamination of BG will occur (which is strengthened by recent literature, see section 4.2), therefore the data of mAb 6 and mAb 5 were assumed to be representative of common BG concentration in DS. Table 9 lists the relevant comparison of the expected BG amount per maximum daily dose (MDD) and PDE. The respective calculations are depicted in section 3.4. Raw data as well as intermediate results of the assessment of criticality of BG contamination in DS are business internal and therefore confidential.

Table 9 Assessment of criticality of BG contamination in DS of all analyzed mAb samples produced at Rentschler Biopharma SE (mAb 5, mAb 6 and mAb 7, respectively). The steps (A) and (B) are business internal information and therefore confidential. To use worst-case approaches, the BG levels found in the analyzed mAb samples were further looked at in the context of the maximum daily dose (MDD) to cover maximum potential contamination of BG (C). Since the expected BG amount per MDD is compared to the PDE, it can be concluded with this formula if there exists toxicological concerns or not (F).

Steps	Levels					Remarks
			mAb7			
	mAb 5[1]	mAb 6[1]	Lot 1	Lot 2	Lot 3	
(A) BG levels in DS in pg/mg	confidential data.					Business internal information. Confidential.
(B) Maximum Daily Dose (MDD) in mg	confidential data.					
(C) BG per day, when MDD is applied in pg/day ((A)x(B))	confidential data.					Worst case approach

(D) BG per day, when MDD is applied in µg/day = expected BG amount per MDD ((C)/10^6)	confidential data.					
(E) PDE_{IV} 3 µg BG per day	3					PDE assessment according to section 3.3.
(F) Comparison of expected BG amount per MDD and PDE ((D)/(E)) = Factor (F)	0.0000[2]	0.0065	0.0021	0.0028	0.0014	Calculation of factor. No toxicological concern due to BG amounts; (F) < 1

[1] Data of mAb 5 and mAb 6 used from UF/DF step (business internal information, confidential). A respective justification is described in the text above.

[2] Calculation of (F) factor resulted for mAb 5 in 0.0000864. Due to better readability of the overall table only four decimal places were reported.

The same approach was also applied to the PDE published in the literature (500 ng per dose; [12]) and is depicted in Table 10.

Table 10 Assessment of criticality of BG contamination in DS of all analyzed mAb samples produced at Rentschler Biopharma SE (mAb 5, mAb 6 and mAb 7, respectively). The steps (A) and (B) are business internal information and therefore confidential. To use worst-case approaches, the BG levels found in the analyzed mAb samples were further looked at in the context of the maximum daily dose (MDD) to cover maximum potential contamination of BG (C). Since the expected BG amount per MDD is compared to the PDE (from MHRA accepted safety dose of BG per day), it can be concluded with this formula if there exists toxicological concerns or not (F).

	Levels					
			mAb7			
Steps[1]	mAb 5[2]	mAb 6[2]	Lot 1	Lot 2	Lot 3	Remarks
(E) Safety dose of BG per day (according to [12]) in µg per day	0.5					From MHRA accepted safety dose

						of BG per day [12].
(F) Comparison of expected BG amount per MDD and safety dose ((D)/(E)) = Factor (F)	0.0005	0.0389	0.0124	0.0165	0.0083	Calculation of factor. No toxicological concern due to BG amounts; (F) < 1

[1] Steps (A) to (D) similar for Table 9 and Table 10.

[2] Data of mAb 5 and mAb 6 used from UF/DF step (business internal information, confidential). A respective justification is described in the text above.

As can be seen in Table 9, the factor (F) in DS or after UF/DF (mAb 5, mAb 6 and mAb 7, respectively) was found to be far below 1. For BG contaminations on DS level (mAb 7, lot 1 to 3; see Table 9) comparable results were found (0.0021, 0.0028 and 0.0014 for lot 1, 2 and 3, respectively). For the data gathered for the mAbs produced at Rentschler Biopharma SE no toxicological concern arose.

The same data was also applied to the PDE, which was published by Barton et al. [12]. This safety dose is six times lower than the PDE derived from external ERT (0.5 µg per day vs. 3 µg per day, respectively). As can be seen in Table 10, for BG contaminations on DS (mAb 7) or UF/DF level (mAb 5 and mAb 6, respectively), the factors for those mAbs in the context of the respective PDE of 0.5 µg per day were also far below 1.

Taken this information all together, the overall contamination of BG on DS level can be considered as uncritical and therefore uncritical for patient safety, since in the context of both PDEs (external ERT and Barton et al. [12], respectively) the calculated factors between the concentration of BG in mAb 5, mAb 6 and mAb 7 were far below 1.

4.2.3 Risk Evaluation

4.2.3.1 Raw Materials and Excipients

Risk analysis revealed a knowledge gap in the context of BG introduction into the process through raw materials (see section 4.2.2). A classification into the critical group was derived

from this data because raw materials and excipients may be a potential source to jeopardize patient safety. Since the knowledge gap leads to an unknown risk, a holistic risk evaluation based on the data available was not possible for raw materials and excipients.

For a general risk assessment (global risk assessment, applicable to a whole company) the consequence for the knowledge gap concerning raw materials and excipients would result into consideration of all raw materials and excipients used after the last bind-and-elute IEX step. Since all these materials would be considered as critical, a project specific risk assessment (which would be based on the general risk assessment, but would only be applicable for a specific project, e.g. one specific mAb development) would be necessary to decrease the amount of raw materials and excipient, which needs to be evaluated. In this project specific risk assessment, a respective test strategy may be implemented for all raw materials and excipients used. Testing on DS level would consider the overall BG contamination in the DS itself and would therefore also include all possible introductions of BG from raw materials and excipients. This may be recommended for specific cases and specific clinical phases. For further considerations on test strategies see section 5.1 and 5.2.

4.2.3.2 Cellulose derived filter

Risk analysis also revealed a knowledge gap in the context of BG introduction into the process through cellulose derived filter (see section 4.2.2). A classification into the critical group was derived from this data because BG introduced from various cellulose derived filter used after the last bind-and-elute IEX step may have a potential to jeopardize patient safety. Since most vendors do not test for BG leaching, this knowledge gap leads to an unknown risk. Therefore, a holistic evaluation based on the data available was also not possible for cellulose derived filter.

Similar to raw materials and excipients, the consequence for a general risk assessment result into consideration of all cellulose derived filter used after the last bind-and-elute IEX step. In a project specific risk assessment, all relevant cellulose derived filter would need to be evaluated. Consequently, testing at DS level would consider the overall BG contamination in the DS itself and would therefore also include all possible introductions of BG from cellulose derived filter. This may be recommended for specific cases and specific clinical phases. For further considerations on test strategies see section 5.1 and 5.2.

4.2.3.3 Criticality of BG contamination in Drug Substance

For both, raw materials and excipients as well as cellulose derived filter, the risk evaluation resulted into a critical classification, since data and knowledge gaps were identified. To identify the criticality of BG contamination on DS level proceeding from all possible entry options in the DS manufacturing process, mAb DS (mAb 7) and mAb UF/DF level (mAb 5 and mAb 6, respectively) were tested and brought into connection with the PDE_{iv} (for PDE assessment see section 3.3). It was seen that the calculated factors between the concentration of BG in mAb 5, mAb 6 and mAb 7 and the PDE were far below 1 (see section 4.2.2). Even though a critical classification was made for raw materials and excipients as well as cellulose derived filter, the overall contamination of BG on DS level can be considered as uncritical and therefore uncritical for patient safety. Consequently, the overall risk of BG contamination can be considered as rather low since the contamination levels of BG on DS level were far below 1. The uncritical risk evaluation can, however, only be made under the assumption that the mAb DS manufacturing process includes a respective bind-and-elute IEX step. In modern process development, sometimes bind-and-elute IEX steps are not included. For that case, a modified risk assessment needs to be done.

4.2.4 Risk Control

4.2.4.1 Risk Reduction

Since the overall risk of BG contamination can be considered as rather low, mandatory risk reduction strategies do not need to be established. However, concerning risk reduction, some of the following points may need to be considered if overall risk of BG contamination may onetime be identified as critical in risk review processes.

<u>Source of raw materials, excipients, and cellulose derived filter:</u>

It may be necessary to screen for all relevant raw materials, excipients, and cellulose derived filter in the manufacturing process in the context of BG contamination. Respective risk reduction strategies may include change in vendors for raw materials and excipients, which certify for respective BG testing. This may also apply to cellulose derived filter, however a change from cellulose derived filter to synthetic filter may also be applicable in certain cases, especially for filter used post bind-and-elute IEX.

<u>Pre-flushing strategies in cellulose derived filter:</u>

As described in section 4.2.1, pre-flush strategies may be useful to prevent and/or reduce BG contamination into the DS manufacturing process. Therefore, if overall risk of BG contamination may onetime be identified as critical in risk review process, pre-flush strategies may be a useful tool to reduce respective BG contamination.

Bind-and-elute IEX:

As described in section 4.2.1, only bind-and-elute IEX have the ability to deplete BG. Therefore, as part of the risk reduction strategy, when setting up the respective purification process, attention should be paid to the use of at least one bind-and-elute IEX step since flow-through IEX do not deplete BG at all.

4.2.4.2 Risk Acceptance

Even though a critical classification was made for raw materials and excipients as well as cellulose derived filter, the overall contamination of BG on DS level can be considered as uncritical and therefore uncritical for patient safety. Consequently, the residual risk coming from potential contamination of raw materials and excipients as well as cellulose derived filters can be accepted. However, testing of BG may be advisable depending on the respective clinical phase. Test strategies for BG are discussed in section 5.1 and 5.2. Risk acceptance decision may change if respective risk review processes detect new knowledge which indicate that the overall BG contamination in mAb DS may increase to a certain point of concern or even exceed the respective PDE_{iv}.

4.2.5 Output / Result of the QRM process

This risk assessment was performed according to the structure of ICH Q9. Risks identified for BG contamination in DS were the use of raw materials, excipients, and cellulose derived filter after the last bind-and-elute IEX step. In the subsequent risk analysis step, the potential contributors were analyzed, and calculations were made to identify the criticality of those potential contributors. In risk analysis, for all identified potential contributors, it was concluded that critical classification should be made in the context of BG contamination properties due to significant knowledge and data gaps and should therefore be considered as a potential source to jeopardize patient safety. Subsequently, real data of BG levels in DS and on UF/DF level were assessed and calculations against the PDE_{iv} were done. It was shown that the overall contamination of BG on DS and on UF/DF level can be considered as uncritical and

therefore uncritical for patient safety, since in the context of used PDEs, the calculated factors between the concentration of BG in used mAbs were far below 1. Consequently, in risk evaluation, the risk for raw materials, excipients, and cellulose derived filter were classified as critical due to the data and knowledge gaps, however due to the uncritically of the overall BG contamination in DS based on real data, the overall risk of BG contamination can be considered as low since this indicates that the process is under control in the context of BG contamination. This results into the acceptance of the identified risks and no mandatory risk reduction strategies need to be established.

5. Discussion

The discussion section encompasses the second part of the first aim (section 5.1), namely the discussion of a respective testing strategy of BG. Subsequently, as second aim (section 5.2), the risk assessment according to section 4 will be discussed into the regulatory framework. In this context, it will be discussed and interpreted, if a respective testing strategy is necessary at different clinical phases and if so, how it should look like. This will also be discussed for the market stage. The third aim (section 5.3) will be focused on the respective regulatory dossiers. It will be discussed and interpreted, if data concerning BG as leachable/impurity shall be included in regulatory submission dossier. Possible regulatory strategies will be discussed, how this data should look like in the respective dossiers and in which dossier section of the CTD granularity is involved. Furthermore, potential regulatory changes potentially coming up in post-approval stages will be assessed (section 5.4). The fourth aim (section 5.5) will take a look in the closer future and will analyze if the regulatory gap of extractables and leachables can be fully covered with regulatory risk assessments. This will also be discussed in the context of the upcoming ICH Q3E.

5.1 Potential testing strategy of BG

In the risk assessment according to section 4, it was shown that the overall contamination of BG on DS level can be considered as uncritical and therefore uncritical for patient safety, since in the context of used PDEs, the calculated factors between the concentration of BG in used mAbs were far below 1. Even though, the risk assessment revealed an uncritical result, recent experiences with authority questions demanded at minimum a summary of the risk evaluation (which would be equal to the outcome of the risk assessment), but in particular for BG an adequate control. The question for an adequate control for BG can be read in conjunction with the WHO guideline for the production and quality control of mAb and related products intended for medicinal use [7], where it is stated that testing for BG should also be considered, particularly if the host cell is known to generate BG or if cellulose filters are used. The passage “should be considered” can be interpreted that a respective testing strategy shall be implemented, unless otherwise justified. Consequently, a respective testing strategy of BG should be implemented. This subsection will only be focused on the testing strategy question on which process stages it should be tested. The next subsection (section 5.2) will discuss the respective testing strategy at different clinical phases and market

application. The risk assessment revealed that the risk for raw materials, excipients, and cellulose derived filter is classified as critical due to the missing data and knowledge gaps section 4.2.5). Some risk reduction strategies were discussed in the results section (section 4.2.4) and to strength the knowledge and therefore the patient safety, considerations for respective testing of BG on those stages may be considered. Consequently, for a potential testing strategy, it should be considered at which step (USP and DSP, respectively) as well as for which materials (raw materials, excipients as well as cellulose derived filters, respectively) it should be tested. Figure 7 shows the overall process (USP and DSP, respectively) of a mAb production in the context of a potential BG contamination in DS after all steps.

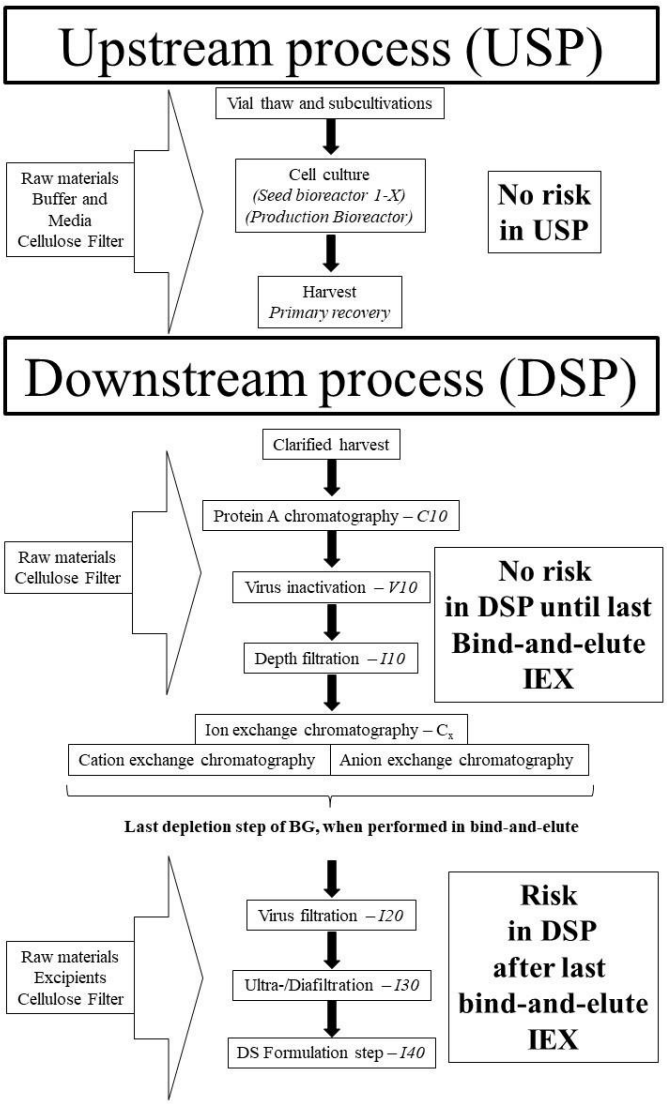

Figure 7 Overall process (USP and DSP, respectively) of a mAb production in the context of a potential BG contamination in DS after all steps. In USP, no risk of BG contamination is expected, since clearance properties in the following DSP are expected to remove all BG content introduced through USP. In DSP, no risk is expected until the last bind-and-elute IEX step, since the clearing properties until the last bind-and-elute step are expected to remove BG content to an acceptable level. A

risk of BG contamination is expected in DSP after the last bind-and-elute IEX step, since no BG clearing steps will be performed after the last bind-and-elute IEX step.

Figure 7 shows, that no risk of BG contamination in DS is suspected from USP and DSP until last bind-and-elute IEX step. This assumption was discussed in the results sections (section 4.2.1). For all steps downstream of the last bind-and-elute IEX step respective tests may be useful. Two different approaches may be feasible (Table 11).

Table 11 Different approaches of testing strategies in the context of BG contamination.

Approach 1: Testing only on DS level	**Approach 2: Testing of incoming goods and on DSP steps as well as DS level**
+ Easy to test + Knowledge of overall contamination + cost efficient	+ broad knowledge of entry options of BG contaminations + easier for risk management (risk control) +/- test strategy may only be applied on specific levels
- Knowledge gap at which step or from which material the BG contamination comes from	- not easy to test, testing strategy may be expanded - not cost efficient

For the sake of simplicity only two different approaches (Table 11) are mentioned in this thesis. However, it must be noted that approach 2 (testing of incoming goods and on DSP steps as well as DS level) may be extended or reduced depending on the respective needs. Approach 1 suggests to only test on DS level. This would facilitate the knowledge of overall contamination, would be cost efficient and easy to test, since only DS samples needs to be taken with this approach. From a regulatory point of view, if the authority request experiences are read in conjunction with the WHO guideline [7] where it is stated that testing for BG should also be considered, it seems sufficient to only test at DS level to have BG contamination adequate in control. However, from a scientific point of view, testing on only DS level may lead to knowledge gaps at which or from which material the BG contamination comes from. This knowledge gap may not be regulatory relevant, if adequate control on DS level is provided, however if failing to meet a specified target for BG in DS testing, then this knowledge gap takes place and require respective risk mitigation actions. This scenario would

not take place if approach 2 is used. Approach 2 would expand the respective BG testing from DS only to incoming goods (which would include, inter alia, raw materials and cellulose derived filter) and on DSP steps, where risk of entry of BG is probably increased, in addition to testing on DS level. This would not only broad the knowledge of entry options of BG contaminations but would also close a respective knowledge gap if the scenario of failing to meet a specified target for BG in DS testing takes place. Therefore, possible required risk mitigation actions could be easier implemented and consequently the overall risk control would benefit. Depending on the process, approach 2 test strategy may only be applied on specific levels, however, these levels need to be defined on an individual basis. Even though approach 2 seem to be the more holistic approach, it also has some negative aspects. Expanding the scope of testing (DS level only vs. incoming good, DSP steps as well as DS level, respectively) will also lead to a more complex test strategy and would therefore require deep process knowledge. This will also drive-up costs (such as testing material, personnel, time and equipment) and will therefore not as cost efficient as approach 1. In comparison, both approaches may be applicable depending on the respective scope (business decision where all variables need to be considered).

Taken all information as well as the recent authority experiences together, approach 1 may be appropriate to meet the regulatory expectations, since a summary of a risk evaluation of BG (e.g., a respective risk assessment, as described in section 4) and testing on DS level only will provide sufficient information to demonstrate that a respective process is in control regarding BG contamination. The next subsection (section 5.2) will discuss the respective testing strategy at different clinical phases and market application and will also take approach 1 and approach 2 (Table 11) into account depending on the respective phases.

5.2 Test strategy in the context of clinical phase I, II, III and market stage

The scope of an application differs depending on the respective clinical phase (Phase I, II and III, respectively) and entry into market through a marketing application. Since drug development and manufacturing processes grow over time, the knowledge of impurities such as BG is rather low beginning of Phase I and will extend to higher knowledges until marketing applications. Further knowledge of impurities may be gained post-authorization. Consequently, the information on impurities will only be limited through a respective IND and IMPD and will expand through the different clinical phases. Deep process understanding will

be gained though PV procedures resulting into BLA and/or MAA. Because of non-existence of internationally harmonized guidance on E&L industry and regulators are uncertain due to lack of clarity regarding such E&L to meet regulatory expectations (as described in section 1). Consequently, regulatory guidelines such as IMPD and IND guideline for biological products [22] [20] as well as ICH MQ4 [28] can only be used to a certain extent.

Clinical phases I and II:

EMA Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials [22] clearly states that PRIs should be addressed in the S.3.2 Impurities section. In the context of PRIs, the EMA IMPD guideline also mentions PRIs such as column leachables. A typical known column leachable, which is generally addressed in S.3.2 Impurities is Protein A, which leaches from Protein A chromatography, the first step in DSP (Figure 3 – C10). In the case of Protein A, it is important to have the process under control, because Protein A is both immunogenic and mitogenic [42], therefore regulators require its clearance to acceptable levels. If this knowledge would be one-to-one transferred to BG as a column leachable, then testing would be recommended from clinical phase I onwards. However, in reality, it is not suitable to do a one-to-one transfer. In that specific case, we should look at the regulatory as well as the scientific background. From a scientific point of view the exact immunological actions and signaling pathway induced by BG are still unclear (section 1) and must be further defined therefore it is whether in scientific nor in industry environment clear to what extent BG should be monitored. From a regulatory point of view, the recent authority experiences were made from market application dossiers (BLA and MAA, respectively). The background of the authority requests was more likely to clearly give a risk evaluation concerning BG. Consequently, from a regulatory point of view, it is not clear whether to test or not during early clinical phases. The risk assessment conducted during this master thesis outlined, that the overall risk of BG contamination can be considered as low, since analyzed DS levels were far below the respective PDE (section 4.2.3). This finding is also strengthened by recent literature (as discussed in section 4). This also needs to be looked in conjunction with the respective clinical phase I. Safety is the main concern, which is addressed with pharmacology and toxicology data. Therefore, DS has been tested, thus impurity profile and potency are known in animals before given to humans. Furthermore, phase I studies have generally a small

number of patients and trial duration been normally short. Those studies are conducted under strictly controlled settings, where adverse events can be monitored [23]. Manufacturing processes will be refined during clinical phases and therefore improved. Since drug development and manufacturing processes grow over time, and knowledge will be gained during the clinical phases, it seems reasonable to not test during clinical phase I/II. However, a respective statement could be made in the IMPD/IND section S.3.2 Impurities, that other potential PRIs will be evaluated by risk assessments regarding their criticality and their potential to pose any risk to patient safety. One such statement could be:

"Other process related impurities than […][1] will be evaluated by risk assessments regarding their criticality and their potential to pose any risk to patient safety. In general, the manufacturing process is developed to sufficiently remove process related impurities. The concentrations of potential process related impurities are expected to be very low. The risk assessments aim to identify and assess those substances, from which risk may remain also at very low concentration in DS. A respective corresponding toxicologically relevant limit is calculated taking worst-case models for depletion and permitted daily exposure (PDE) into account. The respective PDEs will be evaluated by a certified toxicologist."

[1] Name of all impurities assessed for this particular clinical phase.

Similar findings were made for IND phase I applications [20].

As development knowledge grows and further clinical phase II takes place, there might be a need to implement a respective testing strategy, depending on whether your knowledge will come to the point, that Chemistry, Manufacturing & Control (CMC) modifications throughout the IMPD/IND process can affect safety. This includes, inter alia, a change in manufacturing processes that can affect impurity clearance for DS [24]. However, this extremely depends on various factors such as use of contaminated incoming goods, cellulose derived filters as well as on occurring interference in bacterial endotoxin IPC testing (section 1). One such necessity was described by Barton et al. [12], where contamination with BG was observed and safety concerns were made. In general, if a manufacturing process takes place, where no relevant concern arises regarding BG contamination, one may suggest that during clinical phase II, no testing strategy of BG needs to be implemented.

<u>Clinical phase III and PV:</u>

For phase III clinical trial applications, a similar approach can be made as for clinical trials in phase II. Therefore, if no relevant concern arises regarding BG contamination, no testing strategy of BG needs to be implemented for the preparation of phase III clinical trials applications.

During the course of clinical phase III trials, process validation also takes place. Briefly, the term process validation is defined as:

> *"[…] the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API meeting its predetermined specifications and quality attributes" – ICH Q7* [17]

The author wants to emphasize here, that this master thesis does not aim to describe PV principles in detail. The described principles are based on EMA [25] and FDA [26] PV guidelines and will be applied for the objectives of this master thesis. In the course of PV, it should be demonstrated that the process is robust and has the capability to deliver a product of the intended quality [25]. This also includes to prove that BG contamination on DS level is safe and under control. One such prove could be made through clearance studies. Most impurity clearance studies can not be considered in early process design experiments and should be evaluated in the context of the product quality at commercial scale [26]. For those studies, evaluation of selected steps, operating in worst case conditions can be performed to support robustness [25]. Even though in EMAs PV guideline [25] the term "can be performed" is used, worst case conditions represent the most holistic and risk-based approach way to prove the robustness of such process step. Therefore, the author would like to emphasize here to use worst-case scenarios in the context of BG clearance in the process steps. Prior to actual worst-case data analysis, risk assessments may be performed [25]. Such risk assessments could look like the risk assessment performed in this master thesis. Depending on the outcome of such risk assessment, a respective clearance study to prove robustness and the capability of the steps in the context of BG contamination to deliver products of the intended quality can be set up during process performance qualification (PPQ). In general, three to five PPQ batches are used for PV activities, however, this is highly dependable on the respective (scientific) approach to determine the number of PPQ batches [43]. It must be noted that the absence of specific leaching studies may be acceptable for some resins, if appropriately justified [25]. However, as described in section 4, BG not only leach from cellulose derived filters or column

but may also be introduced through various reasons. Therefore, in the context of BG contamination, it is useful to conduct both, risk assessment and clearance studies. Before conducting clearance studies, such as BG clearance studies during DSP, it must be set up a respective PPQ protocol, where test points and adequate analytical methods are defined. A respective test strategy for BG clearance studies on the basis of the risk assessment performed in this master thesis could look like described in Figure 8.

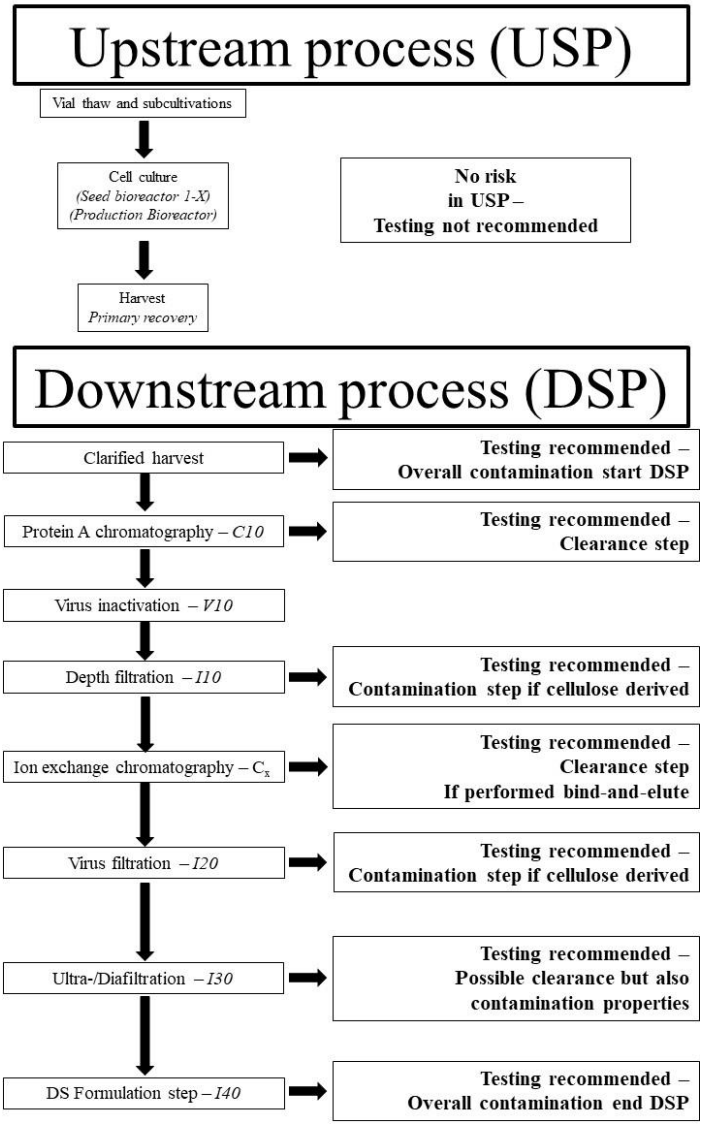

Figure 8 Description of a respective test strategy for BG clearance studies on the basis of the risk assessment performed in this master thesis. Testing of BG contamination is recommended over the full DSP, except of testing on V10, where no clearing and no contamination properties can be excepted (as described in section 4.2.1).

As discussed in section 4, overall contamination on DS level is extremely unlikely coming from USP therefore testing on USP stages is not recommended (Figure 8). For clearance studies, starting point could be the clarified harvest since this is the respective starting point for DSP.

Therefore, to calculate clearance of the upcoming DSP steps, the overall contamination from the start needs to be defined (Figure 8). First DSP step to test for potential clearance would be C10 (Figure 8). The excellent clearing properties of this step are discussed in section 4.2.1. Consequently, as the probably major source of clearing of BG, this step needs to be evaluated in clearing studies. As a probable contamination source if cellulose-derived filters are used, I10 should be analyzed subsequently (Figure 8). I10 has not to be investigated if filters are used which do not compose of cellulose and are therefore no risk for leaching of BG. Next, the C_x steps should be investigated (Figure 8). As discussed in section 4.2.1 the clearing properties of this step are mainly dependent of the respective mode. If performed in bind-and-elute mode, it is highly probable that clearing properties remove BG sufficiently. However, if performed in flow-through mode, it is probable that no clearing properties exist (section 4.2.1). Similar to the I10 step, also the I20 step should be analyzed if the filter is made of cellulose or cellulose derived material (Figure 8). Also similar to I10, I20 has not to be investigated if filters are used which do not compose of cellulose and are therefore no risk for leaching of BG. Clearance but also contamination properties may be introduced through the I30 step as discussed in section 4.2.1. Since it is possible that, if using cellulose derived cassettes, BG is contaminated through I30 step but also, depending on the respective molecular weight of the BG contamination coming from previous steps, cleared through I30. Therefore, testing is recommended on that stage (Figure 8). The overall contamination after DSP needs also to be tested (Figure 8). Comparison between the overall BG contamination at DS level and the respective PDE will then give a statement about the safety in the context of BG contamination and overall if the process is in control regarding BG contamination. For the calculation of the clearance properties of the respective steps, equations such as the following can be used (modified according to [44]):

$$BG\ clearance\ (\%) = 100 * \frac{\frac{Initial\ BG\ concentration}{Intial\ protein\ concentration}}{\frac{final\ BG\ concentration}{final\ protein\ concentration}}$$

It is highly depending on the outcome of the clearance studies how to proceed afterwards. However, applying the knowledge of the recent literature as well as internal data (business internal data, confidential), it can be assumed that the overall contamination on DS level will be far below the respective PDE (as discussed in section 4.2.2 and 4.2.3). Consequently, from

a regulatory point of view, some assumptions can be made of the clearance studies during PPQ and a respective testing strategy for the upcoming GMP batches can be assessed. The assumption can be made that the clearing properties of the DSP is robust and the capability of the process, in the context of BG contamination, to deliver a product of the intended quality in these conditions was proven during PPQ. In general, as described above, a respective PPQ campaign typically consists of three to five batches. Therefore, limited data, especially for the overall contamination of BG on DS level is available. A respective CMC regulatory strategy could assume, that the regulators want to see more data at market application filing until to the point where it can be considered to remove testing of BG on a statistically basis. Therefore, as a consequence of the risk assessment as well as the clearing studies during PPQ campaign, BG content on DS level should be analyzed as additional testing on the upcoming GMP batches intended for either clinical phase III supply or market supply until market authorization. The author wants to emphasize here, that the number of GMP batches for additional testing needs to be defined in interdisciplinary teams, where respective subject matter experts and regulatory affairs decide on the respective regulatory strategy. Taking the respective CMC regulatory strategy, as described above, into account, a statement could be made in the market dossier section S.3.2 Impurities, that clearance of BG was shown, and all data lay far below the PDE and therefore no further testing was derived from these information. One such statement could be:

"[…] clearance of BG was shown in […][1]. All data lay far below the PDE of […][2] as shown in […][3]. Furthermore, additional testing on DS level, as shown in […][3], also demonstrated, that the BG content on DS level lay far below the PDE of […][2]. Concluded from this information, no further testing of BG content will be necessary."

[1] Cross-reference to subsection, where clearance of BG is shown. Typically, cross-reference to 3.2.S.2.5 Process Validation, as shown in section 5.3, will be done.

[2] Cross-reference to subsection, where all data will be depicted in the context of the respective PDE. Typically, cross-reference to 3.2.S.3.2 Impurities, as shown in section 5.3, will be done.

[3] Cross-reference to subsection, where all additional testing on DS level will be depicted. Typically, cross-reference to 3.2.S.3.2 Impurities and/or 3.2.S.4.4 batch analyses, as shown in section 5.3, will be done.

Market submission stage/Post-marketing stage:

How the respective testing strategy should look like is highly dependent on the feedback from the authority during the application procedure and if as well as which feedback was received at this stage (FDA – information request; EMA – List of Questions). From an industry perspective, the respective strategy defined after PPQ (clearance studies in PPQ and testing on DS level on the upcoming GMP batches, see paragraph above) should be conducted until to the point where it can be considered to remove testing on BG on a statistically basis. Consequently, at the end of this strategy, prove of robustness of the process as well as the capability of the process to deliver a product of the intended quality was shown. It was further consistently verified through additional testing of BG content at DS level. This confirms the intended performance of the process to consistently generate the targeted quality of DS in the context of BG contamination (FDA – stage 3 continued process verification; EMA – process verification; [26] [25]).

The next subsection (section 5.3) will discuss if data concerning BG as a leachable/impurity shall be included in regulatory submission dossiers. A regulatory strategy will be discussed, how this data should look like in the respective dossiers and which dossier section of the CTD granularity is involved. Information in this subsection concerning a respective testing strategy will be used to set up high quality dossier sections.

5.3 Compilation of regulatory dossiers in the context of BG

In this subsection, focus will only be made on the CTD granularity of the S sections of the respective dossiers [28]. Furthermore, it will be focused on 3.2.S sections, since this master thesis only discusses its aims to DS level. Since 2.3.S only outlines the body of data in module 3 (3.2.S in that specific case, respectively), this is also excluded from discussions of this subsection.

Clinical phases I, II and III (IMPD and IND):

As discussed in the previous section, a respective statement could be made into the regulatory dossier (section 5.2). However, since risk assessment and real data analyses starting right before, during and after PPQ, no data can be included into the respective regulatory dossiers.

Market application dossiers:

For market application dossiers the presentation of data of BG is highly dependent on the importance of BG for the overall process (Figure 9). As depicted in Figure 9, BG may be mentioned in several dossier sections. The lead information section is represented by 3.2.S.3.2 Impurities (Figure 9). Since BG may be originated from many sources (as discussed in section 4) and are generally recognized as impurities (either coming from cellulose derived filter and are therefore be considered as leachable or as an impurity coming from diverse sources) most information will be depicted in 3.2.S.3.2 Impurities. In 3.2.S.3.2 Impurities the risk assessment including the outcome thereof may be summarized. Furthermore, data of BG content on DS level (depending on the respective testing strategy, as described above, e.g., as additional testing after PPQ) from PPQ batches and onwards should also be summarized in 3.2.S.3.2 Impurities. Using 3.2.S.3.2 Impurities as a lead dossier section, it can be used to cross-reference through the dossier to diverse sections, depending on where information on BG needs to be included (Figure 9).

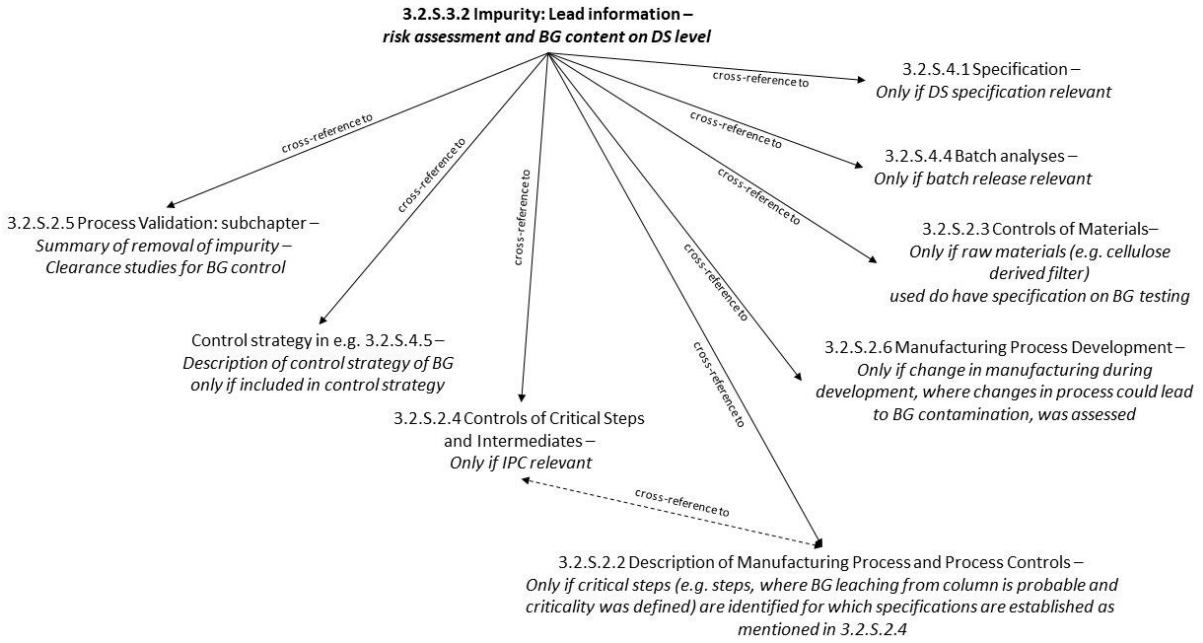

Figure 9 Potential market dossier sections, where information on BG could be included. 3.2.S.3.2 Impurity works as lead information section, from which cross-references through the dossier to diverse sections, depending on where information on BG needs to be included. Cross-reference to diverse dossier section can be made if information seems to be relevant for the respective dossier section. Discussion on when information seems to be relevant in the context of BG is described in the text.

If the testing strategy for BG as described above (section 5.1 and 5.2) is used, then cross-reference from 3.2.S.3.2 Impurities to 3.2.S.2.5 Process Validation – Subsection summary of

removal of impurity – clearance studies for BG control can be made. The information provided in 3.2.S.3.2 Impurities (low BG content on DS level; no toxicological concern, as described in section 4.2.2 and 4.2.3) will be enriched by data from the respective testing strategy during PPQ campaign as described above (section 5.2). In 3.2.S.2.5 Process Validation – Subsection summary of removal of impurity – clearance studies for BG control the data of BG contamination on the respective DSP steps (e.g., in tabular form) and a short statement on the fate of BG during the DSP should be provided. The data provided in 3.2.S.2.5 Process Validation – Subsection summary of removal of impurity – clearance studies for BG control should be used to describe the overall criticality of BG contamination on DS level in 3.2.S.3.2 Impurities. In general, if no concern arose regarding BG contamination on DS level based on data from PPQ campaign and additional testing from PPQ campaign onwards and BG testing will be done as additional testing (as described above, section 5.1 and 5.2), data in 3.2.S.3.2 Impurities and 3.2.S.2.5 Process Validation – Subsection summary of removal of impurity – clearance studies for BG control should be sufficient for market applications.

Further cross-references from 3.2.S.3.2 Impurities to different dossier sections may be possible depending on the importance of BG. The author wants to emphasize here, that the highest probability that data on BG contamination will be included in 3.2.S.3.2 Impurities and 3.2.S.2.5 Process Validation – Subsection summary of removal of impurity – clearance studies for BG control, and only in very specific cases (as described in the following) further dossier sections need data in the context of BG. If BG as an impurity is included in DS specification, then cross-reference to 3.2.S.4.1 Specification is highly recommended (Figure 9, Barton et al. [12] included BG in their respective DS specification, which was accepted by MHRA, however, this was only in clinical phase). If BG is included in 3.2.S.4.1 Specification, then an analytical method as well as its validation is described in 3.2.S.4.2 Analytical Procedures and 3.2.S.4.3 Validation of Analytical Procedures and should be cross-referenced at minimum from 3.2.S.4.1 Specification (not described in Figure 9). If BG analysis is batch release relevant (which would, inter alia, be the case, if BG is included in 3.2.S.4.1 Specification), then data will be provided in 3.2.S.4.4 Batch Analyses and respective cross-references from 3.2.S.3.2 Impurities to 3.2.S.4.4 Batch Analyses should be made (Figure 9). If materials used in the process do have a respective specification where BG testing is included, then cross reference to 3.2.S.2.3 Controls of Materials can be made. If changes in manufacturing process was made

during development and these changes were also assessed in the context of BG contamination (e.g., change of filter from synthetic to cellulose derived filter) then these data will be included in 3.2.S.2.6 Manufacturing Process Development. This data should also be used, if assessed, to strengthen the data derived from PPQ campaign (3.2.S.2.5 Process Validation – Subsection summary of removal of impurity – clearance studies for BG control) and from additional testing on DS level (3.2.S.3.2 Impurities). 3.2.S.2.2 Description of Manufacturing Process and Process Controls as well as 3.2.S.2.4 Controls of Critical Steps and Intermediates may be cross-referenced (also between each other) but only if critical steps (e.g., steps, where BG leaching from a column is probable and criticality was defined) are identified, for which specifications are established as mentioned in 3.2.S.2.4 Controls of Critical Steps and Intermediates (Figure 9). As mentioned in ICH M4(R4) [27] an overall control strategy summary could be placed in several possible locations since there are currently no specific locations defined for control strategy summary in module 3. Overall control strategy summaries may be placed, inter alia, in 3.2.S.4.5 Justification of Specification. Consequently, if BG control is part of the respective control strategy, then cross-reference to the control strategy, e.g., as included in 3.2.S.4.5 Justification of Specification, should be made (Figure 9).

In the context of BG (and also in general for all information included into the respective dossier), the overall goal of the provision of data in the marketing dossier should be to convince the respective authority that the process is robust, under control and the process has the capability to deliver a product of the intended quality and that it will be further consistently verified. Consequently, the regulatory strategy of presentation of BG data is crucial for the success of this specific goal. In the next subsection (section 5.4) it will be discussed if post-approval changes in the commercial production for market supply will have an impact on the information of BG in the respective market dossier on a specific case.

5.4 Post-approval production changes in the context of BG

Changes need to be assessed for their regulatory impact separately per affected region. Regulatory provisions for handling and classification of changes between USA and EU are in general not compatible.

Applicable guidelines for change evaluation in USA:

Products as mentioned in this master thesis are normally specified biological products as defined in 21 CFR 601.2(a) [45]. Hence the following guidelines on regulatory changes are applicable:

- Changes to an Approved Application for Specified Biotechnology and Specified Synthetic Biological Products [46].
- CMC Postapproval Manufacturing Changes for Specified Biological Products to be documented in Annual Reports [47].

In these guidelines, a further elaboration on the different change categories and examples are given. If no suitable examples are given in these guidelines, then the following guidelines which are applicable for certain biological products can be taken into account:

- Chemistry, Manufacturing and Controls Changes to an Approved Application: Certain Biological Products [48].
- CMC Postapproval Manufacturing Changes to be documented in Annual Reports [49].

Changes can be classified in different categories depending on the potential of the change to have impact on quality, safety, and efficacy. Changes can be classified in [48]:

- Prior Approval Supplement (PAS) when changes have a substantial potential to have an adverse effect on product quality [48].
- Changes being effected in 30 Days/Changes being effected Supplements (CBE30/CBE), when changes have a moderate potential to have an adverse effect on product quality [48].
- Annual Report, when changes have a minimal potential to have an adverse effect on product quality [48].

<u>Applicable guidelines for change evaluation in EU:</u>

A variation is a change to the terms of a marketing authorization. The legal framework for handling of variations is established in Commission Regulation (EC) no 1234/2008 [50], amended by Commission Regulation (EU) no 712/2012 [51]. Variations can be classified in different categories, depending on the level of risk to public health and the impact on the quality, safety, and efficacy. Variations can be classified in [50]:

- Type IA variation, when a minor change to a marketing authorization has a minimal or no impact on quality, safety or efficacy of the product [50].
- Type IB variation, when a minor change to a marketing authorization does not require formal approval but must be notified to the regulatory authority before implementation [50].
- Type II variation, when a major change to a marketing authorization has a significant impact on the quality, safety or efficacy of the product [50].

A guideline on the details of the various categories of variations was established [52]. In this guideline the variation categories are further explained and details, how the different variation categories are applied, are given. This guideline is valid for both, biological products as well as small molecules. In its annex the category of variations is assessed with the help of classification schemes. The schemes are assigned to certain topics to make the identification of the relevant information easier.

Post-Approval Change and impact on BG content in regulatory dossiers – example:

Since knowledge of the manufacturing process further increases after approval, post-approval changes in the commercial production for market supply appear on a regular basis. It is therefore important to assess the impact of quality changes on quality, safety, and efficacy. In the context of BG, the most likely change in manufacturing would be a respective change of filter in DSP. For this example, a new depth filter shall be changed in the manufacturing process due to a withdrawal of the used depth filter from the respective supplier. The old depth filter was a non-cellulose-derived type, and the new depth filter is a cellulose-derived filter. The change may have an impact on product quality. This example was chosen because it was the most likely case as well as leaching of BG from cellulose-derived filter was the latest experience with authority requests (section 1). This change will be assessed for EU and USA. The author wants to emphasize here that possible comparability exercises needed for that change according to ICH Q5E [53] will not be discussed in the context of this master thesis. Furthermore, not discussed will be regulatory changes in the dossier for section which need to be revised as well after this change (e.g., Change in 3.2.S.2.2 Description of Manufacturing Process and Process Controls) and do not have (presumably) any relation to BG.

EU:

According to the variation guideline [52] a change in depth-filter would result into a B.I.a.2. “Changes in the manufacturing process of the active substance” change (Figure 10).

B.I.a.2 Changes in the manufacturing process of the active substance	Conditions to be fulfilled	Documentation to be supplied	Procedure type
a) Minor change in the manufacturing process of the active substance	1, 2, 3, 4, 5, 6, 7	1, 2, 3	IA
b) Substantial change to the manufacturing process of the active substance which may have a significant impact on the quality, safety or efficacy of the medicinal product			II

Figure 10 Potential variation possibilities when changes in the manufacturing process of the active substance are made. Abstract according to the EU variation guideline [52]. Conditions to be fulfilled are not depicted in this figure but can be found in the respective guideline.

It must be first decided, if this change is considered to be minor (a) or substantial (b). The condition that needs to be fulfilled that this change could be considered as minor (a) is, inter alia, that the active substance is not a biological substance [52]. Since this master thesis mainly considers mAbs, which are biologicals per definition, this condition is not fulfilled for this specific change. Consequently, the change in depth filter needs to be considered as substantial (b) and needs to be changed with the type II variation procedure (Figure 10). As stated out in the variation guideline [52] the application must contain the elements listed in Annex IV of the Commission Regulation (EC) no 1234/2008 [50]. One of such elements are:

- “Supporting data relating to the proposed variation.” [52]
- “Update or Addendum to quality summaries […] as relevant. […]” [52]

In the context of BG contamination, if clearance studies were performed during PPQ and it was also investigated with the old depth filter, then it may be necessary to perform such studies also for the new depth filter. However, it may be sufficient to show that BG contamination on DS level does not exceed the levels with the old depth filter and no toxicological concerns arise using the new depth filter. This must be defined in the respective regulatory strategy for this type II variation. In general, one would suggest performing head-to-head comparability of the use of both filters and provide both datasets as supporting data in the respective type II variation application. Depending on the initial dossier and the

outcome of the implementation of the new depth filter an update or an addendum to quality summaries may be necessary in the context of BG.

USA:

According to FDAs Guidance for Industry – Chemistry, Manufacturing, and Controls Changes to an Approved Application: Certain Biological Products [48] special considerations need to be taken into account on manufacturing changes. A respective manufacturing change, such as the change in depth filter as described above, should be reported prior approval as a PAS when the change has a substantial potential to affect product quality. One may assume that the change in depth filter may have a substantial potential to affect product quality. In appendix 3.2.S.2.2 Description of Manufacturing Process and Process Controls – Change in the Drug Substance Purification Process it is clearly stated that a revised purification process (such as a change in depth filter) must be report prior approval as a PAS (Figure 11; [48]).

PRIOR APPROVAL (PAS)
New or revised purification process (e.g., change in the resin or filter material, loading scale, column size, or elution rate of a chromatographic column).

Figure 11 Potential change possibilities when changes in the DS purification process are made. Abstract according to [48]. Conditions for a PAS are depicted in this figure.

In this specific case, the old non-cellulose derived depth filter will be changed into the new cellulose-derived depth filter. This can be considered as a change in the filter material and consequently as a revised purification process which needs PAS. A PAS must be approved by the FDA prior to distribution into the market according to 21 CFR 601.12(b). Submissions under 21 CFR 601.12(b) shall contain, inter alia, a description of the methods used, and studies performed to evaluate the effect of the change as well as the data of the studies [54]. Consequently, a similar, but not equivalent, situation as described above for EU type II variation submission can be concluded. Since FDA is very data-driven, it tends to work from the bottom-up approach to arrive at a decision. In contrast, EMA reviewers tend to take a more top-down approach. Consequently, for PAS, it may be advisable to adapt the bottom-up approach and include more data and statistical approaches into the PAS. It may be necessary to have comparability protocols to assess the effect of the filter change on product

quality. However, this may depend on the respective regulatory strategy, since, if approved, the comparability protocol may justify a less burdensome reporting category [48].

5.5 Outlook – Extractables and leachables in the (nearer) future

The regulatory gap of E&L is a complex issue that requires careful consideration. As discussed in section 4 of this master thesis, regulatory risk assessments can be helpful in identifying potential risks, however it is rather unlikely that they can fully cover the regulatory gap of E&L. This can be illustrated by a few points. First, E&L encompass a range of potential substances (section 1). Consequently, the regulatory gap leads to the necessity to perform risk assessments to every single potential substance (or at least for specific groups of substances). From the industry perspective, this costs many resources, including human as well as technical, which ultimately is a respective cost driver. From a regulatory perspective, this would result into many risk assessments, which may fulfil the requirements in the sense of ICH Q9 and the risk-based approach, however, there would be no consensus for risk assessments for E&L in detail. This is also addressed in the final concept paper of ICH Q3E [15]. Second, neither identification, qualification nor reporting thresholds are available and safety assessment are only possible with difficulty due to lack of exposure limits of E&L [15]. Currently, for example for derivation of a PDE, a procedure may be applied that is based on the method as recommended in the Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities and described in the Annex 3 of the ICH Q3C [55] [56]. For respective assessments of E&L such thresholds need to be implemented. Third, downstream implementation of the respective control strategy after the risk assessment rather resembles more of a maze than an enlightened path. This is mainly caused by the lack of aligned E&L guidance framework. Consequently, such risk assessments as performed in this master thesis and the downstream implementation of the respective control strategy of the E&L in question as well as the respective regulatory strategy during clinical development as well as filing and post-approval stages are only gap fillers until the establishment of ICH Q3E.

6. Summary

Existing uncertain regulatory environment concerning E&L leads to a need to assess E&L in a risk-based approach to walk through this maze and built a clearer path. BG have been found to have potential immunogenicity risk in biopharmaceutical products and are recognized as contaminants. Recent experiences with market dossier applications triggered questions from authorities on BG such as information on leachable studies and/or summaries of the risk evaluation on BG, which should be considered as potential leachable from the use of cellulose filters in the manufacturing process.

Since those experiences called for adequate control strategies of BG in biopharmaceuticals, this thesis aimed to regulatory risk assess BG. The lack of aligned regulatory guidance framework made it indispensable to use general quality risk management approaches according to ICH Q9.

The first aim encompassed the development of a control-strategy of BG that will be (most-likely) accepted by the authorities at market submission stage. This included a risk assessment according to ICH Q9 as well as the development of a testing strategy of BG. Risks identified for BG contamination in DS were the use of raw materials, excipients, and cellulose derived filter after the last bind-and-elute IEX step. For all identified potential contributors a critical classification was made due to significant knowledge and data gaps and were considered as a potential source to jeopardize patient safety. Interestingly, real data calculations against a PDE revealed factors which were considered as uncritical for patient safety. Consequently, the overall risk of BG contamination was considered as low, which indicated that the specific processes under investigation were under control. Two different approaches of testing strategies for BG were discussed. Approach 1 included testing only on DS level, whereas approach 2 included testing of incoming good and on in-process steps as well as on DS level. Both approaches may be applicable depending on the scope. However, approach 1 was chosen to be more appropriate to meet the regulatory expectations, since risk-assessment and testing on DS level only will provide sufficient information to demonstrate that a respective process in under control.

As second aim, the risk assessment was discussed, interpreted, and embedded in the regulatory framework, if a testing strategy is necessary at different clinical phases and market

stage. It was shown that it is reasonable to not test during clinical phases I and II. Similar findings were made for clinical phase III. It was shown that during PV, it should be demonstrated that the process is robust and has the capability to deliver a product of the intended quality, which also included to prove that BG contamination on DS level is safe and under control. Therefore, a testing strategy was elaborated, which included both approaches from the first aim. It was further shown that approach 1 can be conducted until to the point, where it can be considered to remove testing on BG on a statistically basis. This was discussed to be used until market application stage.

The third aim focused on regulatory dossiers. It was shown in which S sections of the CTD granularity BG should be discussed. Since risk assessment and real data analyses starting before, during and after PPQ, a respective statement was suggested for clinical phases I, II and III. For market application dossiers suggestions were made, where information on BG could be included and how this information can be cross-referenced between the sections. Furthermore, it was discussed if changes in the commercial production for market supply could have an impact on the information of BG in dossiers. Potential regulatory changes for EU and USA were assessed and which form of change must be submitted were discussed.

The fourth aim looked in the closer future. Regulatory risk assessments will be helpful in identifying potential risks. However, risk assessments, and the implementation of control strategies of the E&L in question as well as regulatory strategy during clinical development, filing and post-approval stages were identified as only gap fillers until the establishment of ICH Q3E.

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Annex I

Literature research with PubMed® (first table) and Google Scholar (second table). The Boolean operator "AND" was used. It is shown which word combination were used and how many numbers of hits were found. After reading of all abstracts, the quality of hits was categorized in "usable" and "not usable". To be eligible as "usable" in the context of this master thesis, the respective publication in question should be either a relevant publication with published data on the fate of BG or a review with a summary of relevant publications.

Combination	***Number of hits***	***Quality of hits***
((beta glucane[Title/Abstract]) AND (contamination) AND (downstream)	3	[29] [31] [38]
((beta glucane) AND (downstream)) AND (biopharmaceutical)	83	[29] [32] [31]
((beta glucane) AND (impurity)) AND (downstream)	3	[29] [31]
(beta glucane[Title/Abstract]) AND (downstream[Title/Abstract])	63	[29] [32] [31] [38]
((beta glucane) AND (chromatography)) AND (process)	126	[29] [32] [38] [37]
((beta glucane) AND (membrane)) AND (downstream)	38	[29] [31] [38]
((beta glucan) AND (clearance)) AND (downstream)	9	[29] [32]
Overall publications after deletion of doubling publications: 5 publications		

Combination	***Additional publications***
((beta glucane[Title/Abstract]) AND (contamination) AND (downstream)	[12]
((beta glucane) AND (downstream)) AND (biopharmaceutical)	[39]
((beta glucane) AND (impurity)) AND (downstream)	No additional publications
((beta glucane) AND (chromatography)) AND (process)	No additional publications
((beta gluicane) AND (membrane)) AND (downstream)	No additional publications
((beta glucan) AND (clearance)) AND (downstream)	No additional publications
2 additional publications were found in Google Scholar	

Erklärung

Hiermit erkläre ich an Eides statt, die Arbeit selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Laupheim, 06.03.2024

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