AfA Merck Biodevelopment SAS/ FUJIFILM Diosynth Biotechnologies UK Limited
Submission number: ZN562229-06
Communication number: AFA-C-2114484396-36-01/F
Substance: 4-(1,1,3,3-tetramethylbutyl)phenol, ethoxylated, EC number: -
Clarification on RAC/SEAC questions

RAC questions

1. The application states "As release of Triton X-100 to the environment can be excluded, no risk for the environment by the continued use of the substance in the production of G-CSF is identified"

General remarks by the applicant:

In preparation for the implementation of routine commercial manufacturing, a complete review of our operations resulted in a few operational changes in 2019:

- Implemented (2019): handling of opened bottles of Triton X-100, as described in the submission and detailed below.
- Upcoming (2020):
    - sampling of incoming Triton X-100 at goods receipt, and subsequent identity testing (regulatory requirement[1] for commercial production) – description below;
    - Additional flush of at least 200 L of water prior to initiating the CIP cycle in order to ensure maximum recovery of Triton X-100 from the equipment.

1a) Please can you provide further information to justify that releases can be completely excluded? Please provide measured data to back up claims with regards to 0 releases into all the environmental compartments. Please include contextual information such as: where the monitoring was done, how often, what activity was taking place, etc.

Due to the pursuit of the zero-release strategy, the design of the facility and the production operations were adapted to exclude discharge of Triton X-100 during process operations. Consequently, no measurements have been conducted to date.

A detailed overview of the process at the Martillac site is given below:

- The process effluents containing Triton X-100 [redacted] maximum concentration) are collected in the "toxics effluents" system, which is an independent and entirely segregated waste collection system. The system is hard-piped and directs all "toxic" effluents to a single [redacted] m^3 vessel, placed in a dedicated retention to avoid release of potential toxic spills (e.g. tank failure) to the environment. The entire content of the toxics waste vessel is periodically collected and shipped off-site to be incinerated.
- Process – Washing of inclusion bodies:
    - The [redacted] Triton X-100 buffer is used to wash inclusion bodies. About [redacted] L of inclusion bodies are suspended in about [redacted] L of [redacted] Triton X-100 solution. The inclusion bodies are centrifuged, and the liquid fraction is directed to the toxics waste system, while the inclusion bodies are washed again with the [redacted] Triton X-100 solution (same ratio as before). The centrifuged inclusion bodies are then subjected to an additional 6 successive washes which do not contain any Triton X-

[1] Regulatory requirements include for example cGMP requirements, FDA regulations, EMA GMP requirements ICH Q7.

100 (same procedure as before). All the process effluents (i.e. the liquid fractions from the centrifugations) are collected in the toxics waste system. This ensures a dilution factor of 1'771'561-fold (11^6-fold) of the Triton X-100 concentration along the inclusion body washing process, resulting in a Triton X-100 concentration of about 10 µg/L in the last supernatant sent to the toxics waste system.

- The inclusion bodies are then concentrated further via batch concentration – the supernatant is discarded into the toxics waste system.
- The inclusion bodies are then subjected to solubilization and refolding, resulting in an additional 1000-fold dilution of the Triton X-100 in the process fluids (i.e. around 10 ng/L). The material is then directed to a chromatography purification step – all the effluents generated at this stage (1000 L flow-through from column and 100 L un-used load material) are directed to the toxics waste effluents.

- Preparation of the Triton X-100 containing buffer:

  The equipment used for the preparation and transfer of the [redacted] Triton X-100 buffer is thoroughly drained by compressed air purging into the toxics waste. The equipment is designed for complete drainage (electropolished stainless steel, no low points) which ensures that essentially no liquid residues remain on the walls of the equipment. Before initiation of the cleaning-in-place (CIP), the residual liquid on the surface of the equipment ([redacted] L preparation tank, transfer line, flexible hose and in-line filter) is rinsed twice with water into the toxics waste system. Residual Triton X-100 is further removed prior to initiating the automated CIP cycle by air purging (drainage still directed to toxic waste).

  Based on the water adsorption capacity of electropolished stainless steel as generally accepted in the scientific literature, it is estimated that the amount of liquid still present in the system after the air purge is between [redacted] ml. To account for potential uncertainties related to connections, valves, O-rings, etc., an additional "safety factor" of 10^2 is added. Thus, a residual volume of [redacted] ml of the Triton X-100 buffer [redacted] is assumed to be retained in the system, representing 0.4-4 g of Triton X-100 left on the surface of the system after the air purge in a conservative, absolute worst-case. The system rinse is performed with 400-600 L of water. The concentration of Triton X-100 is therefore between 0.7 and 10 mg/L in the rinse. This rinse water is flushed to the toxics waste. As a worst-case estimate, after this flush [redacted] mL of the rinse water (0.7 to 10 mg/L) is present in the system, corresponding to 14 to 2000 µg of Triton X-100. An additional rinse of more than 200 L of water is performed, leading to a Triton X-100 concentration below 0.07 to 10 µg/L in the rinse. This second rinse water is also flushed into the toxics waste. This means that less than 1 ng to 2 µg (worst case) is present in the system prior to initiating the CIP cycle. The CIP cycle starts with a water pre-rinse (volume 200 L) which is directed to the "pH system", the Triton X-100 concentration is therefore estimated to be below 10 ng/L – basically close to or below the limit of detection of the analytical method of 10 ng/L. Therefore, no analytical measurements were so far conducted.

  - Please note: Possible sampling and analytical confirmation of Triton X-100 in the pre-flush effluent will be possible during the next production run which is planned for [redacted].

[2] Please note that the safety factor is added although connections, valves, O-rings, etc. are designed for full drainage.

For the future production in Billingham it is confirmed that the same risk management measures will be implemented to safely prevent emission of Triton X-100 to the environment:

- Completely segregated waste drain for liquid Triton X-100 waste which feeds into a waste tanker for subsequent off-site treatment by incineration;
- All effluents from washing of inclusion bodies will be directed to the segregated waste drain for Triton X-100;
- Pre-rinsing of equipment prior to initiating CIP cycles with collection of effluents in the segregated waste drain for Triton X-100.

1b) All equipment rinses are collected in the special effluent, but nothing indicated about "glassware", e.g. in in the initial step Triton X-100 is manually transferred to 5L beakers. How are those beakers (and any other similar type of equipment) handled? Are they rinsed with the rinse collected and incinerated, are the equipment themselves collected and incinerated?

For the preparation of the [redacted] Triton X-100 buffer solution, the Martillac production uses two 5 L plastic beakers (see CSR, figures 4 to 6) and a smaller one for the final adjustment of required Triton X-100 volume. These beakers are single-use plastic equipment and are disposed of as toxic waste after use without any pre-cleaning. The beakers are placed in specific chemical waste containers (red plastic bins). Please note: In any handling steps involving Triton X-100 no glassware is used that would require pre-rinsing and collection of additional effluents.

In addition, a plastic bottle is used to collect the Triton X-100 solution flushed from the air purge of the 0.22 µm filter prior to the transfer of the Triton X-100 buffer from the buffer preparation area to the USP area (see CSR, chapter 9.1.1.5.8., p. 36). This plastic bottle containing the liquid collected during the flush procedure is tightly sealed and placed in a yellow bin (biological waste). Red and yellow bins are transferred to the waste bungalows. The bins are subsequently picked-up and sent to incineration by a qualified contractor (see internal SOP EHS-OPER04-WI01: Tri des déchets).

In Billingham, single-use plastic beakers and containers that have been used for handling (undiluted) Triton X-100 will be disposed of as hazardous waste via incineration at an approved external waste treatment facility.

1c) It is also stated the process-related wastewater stream containing Triton X-100 is strictly separated from the remaining wastewater streams of the facility and the sewerage by technical means. Please explain and give examples (pictures) how this is achieved.

Separation of the Triton X-100 containing wastewater stream is ensured by engineering measures, i.e. a separate piping system of production and cleaning processes which involve Triton X-100:

In Martillac, the "toxics effluents" system is an independent and entirely segregated waste collection system. The system is hard-piped and directs all "toxic" effluents towards a single [redacted] vessel, which is located within a dedicated retention to avoid release of potential toxic spills (e.g. from tank failure) to the environment (see Figure 1):

Figure 1: [redacted] surge tank in a concrete retention area under floor level, pumping system to lift toxic effluents to main storage tank, [redacted] toxics effluents main storage tank, with dedicated retention (double wall with level sensor).

Each production suite is equipped with ports and drains that are solely connected to the "toxics" effluents system, i.e. these drains/ports are not shared with any other waste systems (e.g. biologics or pH).

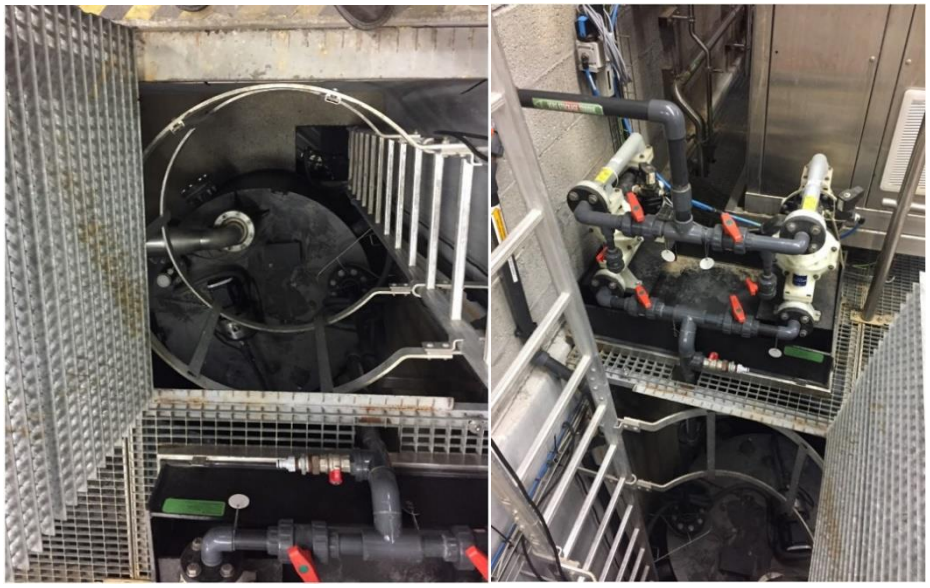

Figure 2: The [redacted] surge tank in its underground concrete retention and the pumping system

Figure 3: Picture of the [redacted] toxics tank in its retention

The entire content of the toxics waste vessel is periodically collected by a qualified supplier and shipped off-site to be incinerated.

The Billingham production will be designed analogously to Martillac with the same level of separation of the waste collection systems. In addition, Fujifilm, Billingham, have previous experience with handling large volumes of segregated waste. The pipework route from the waste holding tanks to the waste tanker (external to the facility) is checked before transfer of waste can begin. Two operators are required to direct the waste to the tanker, with GMP document sign off and visual confirmation that the waste is entering the tanker is required. Waste water is then transferred to a road tanker and taken to an approved waste handling facility.

1d) Please give details on the storage of the solutions used in the process.

Martillac:

1. The Triton X-100 is delivered to the warehouse in boxes, each containing 4 bottles of 2.5 L. After un-crating, the Triton X-100 boxes are stored in the warehouse of the site centralized logistic centre. The warehouse is environmentally controlled (temperature and humidity), with a restricted access. The Triton X-100 bottles are stored in the warehouse in their original primary and secondary packaging (bottles in boxes), in a room dedicated to chemicals (remaining in the dark), until transfer to the buffer preparation area in Manufacturing.

2. Since 2019: For each lot of Triton X-100 delivered √N + 1 (N representing the total number of bottles delivered) are sampled in the dispensing area for identity testing. All the bottles opened for testing must not be stored and used later for processing (see point 1-j). These bottles are set aside and transferred to the toxic waste bungalow for subsequent pick-up and incineration by a qualified contractor.
3. The bottles are transferred to Manufacturing. After unpacking, the bottles are placed in a retention area in the materials air lock and subsequently transferred to the buffer preparation area. In the buffer preparation area, the bottles are stored in a chemical cabinet, in a retention.
4. A [redacted] stainless steel tank is used for the preparation of the [redacted] Triton X-100 solution, and also for storage until use in the process.

Details on storage regarding the future production in **Billingham** cannot (yet) be provided.

1e) Please describe the leakage and spill management in place (e.g. also during manual handling activities).

In Martillac, the operator wears appropriate personal protective equipment (gloves, mask) during the entire cleaning operation. Each process area is equipped with kits designed to absorb and clean up potential spills. Single-use absorptive packs, available in all areas of chemical use, are first placed around and on the spilled liquid. A specific work instruction explains how to act in case of a chemical spill (internal SOP WI-EHS-447-Reflexe Deversement-001) - in addition to the training about chemical risks delivered to all new employees. Once the liquid has been absorbed, the contaminated area is then wiped clean with cloth wipes wetted with detergent/disinfectant solution. The contaminated absorptive packs and wipes are placed in red plastic bins (dedicated to the chemical waste). The closed bins are then transferred with a trolley (equipped with a retention tray) to the chemical waste bungalow (also equipped with a retention area). The bins are then stored in a specific dedicated retention pallet which is directly transferred to the waste pick-up truck. The transportation delivers the waste to the qualified incineration plant on the same day. During all the waste transfer from the plant until the incineration, the traceability of the waste is recorded in a regulatory document. Once the treatment is done, a copy of the completed document is forwarded to the EHS department.

In Billingham, spill containment kits are located in the manufacturing facility which, once used, are disposed of as hazardous waste (by offsite incineration). In order to prevent spills reaching the general waste system, floor drain sealing caps are inserted into all relevant suites. Drain closure is confirmed via GMP documentation sign off. During transit, the undiluted Triton X-100 will be bunded at all times.

At both sites, any potential emissions from tank leakage (not envisaged) and spills from coupling and uncoupling activities (including road tankers collection liquid Triton X-100 for destruction) to the environment are safely prevented by sealed flooring, retention systems, underneath tanks, and the possibility to pump spills back to the respective hazardous waste tank.

1f) How long is the carrying distance of the solution components?

Martillac

Transfer → Warehouse to dispensing area for sampling of raw material: less than [redacted] (2.5 kg per bottle transferred)

Transfer → Warehouse to Production materials airlock: less than [redacted] (up to [redacted] kg of Triton X-100, in separate boxes)

Transfer → Production Materials airlock to buffer preparation: less than [redacted] (up to [redacted] kg on a kart in an appropriately sized retention)

Buffer Preparation → from dispensing hood to [redacted] buffer preparation tank: less than [redacted] (manual transfer in a in a plastic beaker)

Billingham:

Undiluted Triton X-100 is dispensed from a warehouse, contained in a bunded plastic crate and transferred via forklift truck approximately [redacted] to the manufacturing facility. Once inside the facility it is taken via lift to the buffer preparation suit. This distance is approximately [redacted]. Fujifilm also have an EHS SOP that details the procedure for transporting liquids across the site.

1g) At several steps of the process toxic waste drain collection is mentioned. Please describe in more detail the exact steps when toxic waste drain is used. How are the discard activities done into the toxic waste network? (PROC1, PROC 28) Give pictures of the toxic waste drain collection points at the site.

For a detailed description during which steps the toxic waste drain/system is used please kindly refer to the answer to Q1a.

Examples of the "toxic drains" in the USP process area are shown in the pictures below (see Figure 4). The toxic effluents network is segregated from the other effluent systems and the draining ports are all clearly identified. The proper use of the different effluent systems is ensured, and the selection of the adequate effluents system for a given process operation is imposed in the manufacturing batch documentation.

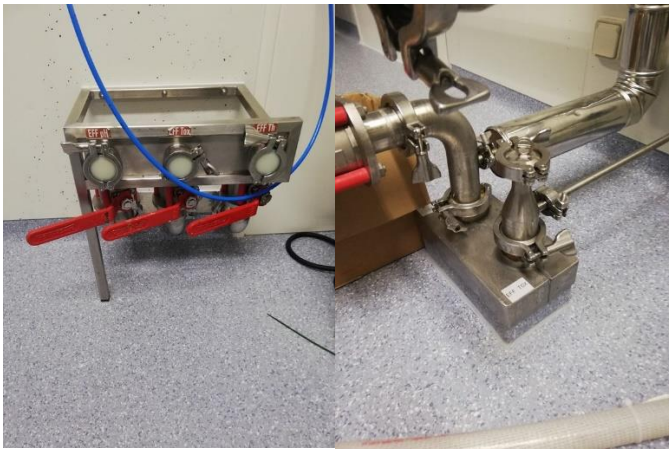

Figure 4: "toxic drains" in the USP process area

1h) It is stated that the effluents from the automated cleaning phase are collected in the pH effluents network and after the pH adjustment the effluent is collected in the sewage system and sent to municipal lagunage. Please provide measured data after the pH adjustment phase to show that the effluents are not contaminated by the substance (or degradation substance).

After complete transfer of the buffer solution to the USP area, tank and transfer line are completely cleared from Triton X-100 (pressurized air, rinsing with water). Except for the "calculable" small residues (please kindly refer to Q1a) no Triton X-100 is released to the pH effluents network and/or the lagunage.

The relevance of a direct monitoring of the Triton X-100 out of the pH system effluent is subject to caution for several reasons:

- Several operations are carried out simultaneously in the plant, each susceptible of generating "pH effluents" that are continuously pH adjusted and discharged to the waste water system and lagunage: CIP and equipment cleaning effluents, excess water from WFI/WPU loops, reverse osmosis "brine" from the purified water production systems, condensed vapours, effluents from the process, etc. … A total of up to [redacted] m^3 of effluents may be generated every day. The few µg of residual Triton X-100 sent to the pH effluents per run will therefore be instantaneously diluted into cubic meters of effluents before release to the waste water system and ultimately the lagunage.
- The discharge of effluents is continuous during a production phase. The residence time of the wastewater inside the pH neutralization tank is therefore variable (depending on the instantaneous flow rate of the effluents) - a spot sampling for the testing of Triton X-100 is therefore likely to be insufficient for a proper characterization of the release to the environment.
- Considering the volumes of "pH effluent" generated on the facility, the peak concentration of the Triton X-100 in the effluent of the site to the lagunage is likely to remain far below the detection limit of the analytical method, regardless of the timing of the sample collection.

The sensitivity of the analytical method developed for the detection and quantification of the Triton X-100 is insufficient (LOQ = 10 ng/L). The estimated concentration of Triton X-100 in the effluents directed to the "pH effluents" is below the LOQ, and even lower in the plant effluents directed to the lagunage.

1i) Explain in more detail the manual rinsing activity of the filters under PROC28 with regards to the risk to workers and surrounding environment.

The evaluation of any potential health risks for workers is not relevant within the framework of this authorisation application, as outlined in chapter 5 of the CSR. However, due to the strict measures and the prescribed PPE (mouth guard, protective clothing, gloves) for the production of pharmaceuticals under cleanroom conditions worker exposure is well controlled.

Filter washing is conducted under controlled conditions. The filter is rinsed a first time along with the buffer preparation tank, the transfer line and the flexible hosing. After draining of all the line elements with compressed air, the integrity of the filter is checked by connecting the filter to *Integritest* equipment.

The Triton X-100 containing effluent from this process step is directly drained to the toxic waste network. Thus, a release of Triton X-100 from filter washing can be excluded.

Workers always work with gloves during this activity and wear protective clothing at any time.

1j) Under waste treatment section 9.1.2 it is mentioned that opened bottles will not be preserved for the following runs/campaigns anymore and any opened bottle will be properly discarded. Please explain since when this practice is taken up?

This practice was taken up since the 2019 production campaign. During an investigation initiated in 2018, it was discovered that the Triton X-100 is subject to auto-oxidation (e.g. after exposure to air or light) – this process generates peroxides which may result in oxidation of the API. Consequently, it was decided that only intact bottles of Triton X-100 could be used for the process. Previously opened bottles are set aside and picked up by a qualified supplier for incineration.

2) How "analogous" are the sites? Tonnage for Martillac is based on number of production runs, while Billingham is based on number of batches, and "scaling-up" is indicated, meaning far larger amounts are used per run/batch, but with much fewer batches leading to a similar overall tonnage. Using far larger amounts means that an emission/spill from a single batch is likely to be much larger.

Both production sites are considered analogous in terms of handling and production steps that involve Triton X-100. Moreover, at both sites Triton X-100 is completely recovered from the production process to prevent releases to the environment. Triton X-100 is/will be collected for subsequent destruction by incineration. Thus, releases of Triton X-100 will be null for processing phases (and only small calculated residuals in CIP phases as shown earlier), irrespective of the exact tonnage used at the sites.

In Martillac, the maximum amount of Triton X-100 potentially susceptible to spillage is the equivalent of about [redacted] kg of pure product, or the equivalent of about [redacted] L of a [redacted] solution depending on the process stage at which the spill occurred. At the Billingham site, [redacted] L to max. [redacted] L of pure Triton X-100, corresponding to [redacted] L of [redacted] solution could be spilled in a worst-case. Both sites have implemented leakage and spill management measures for accidental spills in production and loading of road tanker during coupling/uncoupling to Triton X-100 wastewater tanks (see also question 1e). Thus, emissions of Triton X-100 to the environment from leakage and spill can safely be excluded, irrespective of the actual amount of Triton X-100 used/handled during the production of one batch.

3) Is it correct to assume that there are no gloves illustrated at Figure 4 page 33 of non-confidential CSR but they are used as PPE as it is stated on page 32 under section 9.1.1.4?

Figure 3 of the CSR shows the general gowning/protective equipment required **to enter** the general production areas (e.g. corridors) and specific processing suites (buffer preparation and USP in the figure). **Access** to USP and buffer preparation does not require gloves. However, our procedures also clearly state that **any intervention on the process equipment (e.g. connections, disconnections) or handling of materials** (raw materials, product) in USP or Buffer prep. **requires the use of gloves (ad minima) and adequate protective equipment** – this protective equipment could include an additional pair of gloves (type of glove depending on operation performed - see figure 4), full face shield, respiratory protection (see figure 4), chemical apron ….

4) Please provide non-confidential ranges of substance concentration limits and tonnages used (e.g. batch sizes and number of batches, production runs, etc.).

Non-confidential ranges:

- Substance concentration in buffer: ≥ 0 to ≤ 10 % (v/v)
- Tonnages: 0–1 t/a (for both sites)
- Batch size: 10–250 kg Triton X-100
- Batch number/number of production runs: 1–50

5) RAC takes note that you claim to emit 0 kg of 4-tert-OPnEO per year. RAC may recommend conditions to ensure 0 emissions. If that is the case, would anything, in your view, prevent you from implementing such a condition? Please also provide an estimation of the costs that would be incurred.

We cannot guarantee an absolute "0 emission":

The process is performed in batch mode (discontinuous) and is subject to extremely strict sanitary requirements. Some of these requirements demand stringent cleaning procedures for all pieces of equipment between production runs. Calculated residual amounts of Triton X-100 present on the surfaces of the processing equipment after draining and rinsing is picked up during the cleaning procedures. Consequently, undetectable (< LOQ of 10 ng/L) yet "calculable" Triton X-100 levels might be released in the effluents of the plant. At this point, it is estimated that less than 30-40 µg of Triton X-100 will be released to the environment during each routine production run. The recovery of these small amounts would require extensive re-engineering of the effluents collection and CIP systems, imposing a temporary shutdown of the facility. Such a shutdown is not possible at this stage:

- The beginning of the commercial production is planned for [redacted]. An additional shutdown period at this stage of the project would necessarily delay the commercialization of the product – considering peak sales estimated at [redacted] M€ per year, the estimated loss of revenue for FK associated to the delay would represent [redacted] M€ per calendar month of delay.
- The modification of the facility would require CAPEX investments that are not available this year in the Merck budget and have not been planned [redacted] – the amount would remain to be confirmed.

The "idle capacity" costs in Martillac associated to such a shutdown exceed [redacted] M€ per month (20 open process days) of shutdown. No revenue stemming from the CMO activities performed on behalf of FK (on average, about [redacted] M€/month without production) can be expected during the shutdown, therefore yielding an additional loss of [redacted] M€ per month [redacted]

SEAC questions about the Analysis of Alternatives:

1. Regarding the AoA, has the production of the IBs been evaluated using different means e.g. cell lines, which could circumvent the problem of endotoxins present on the membrane of E. Coli?

No, as such considerations are clearly not conducive to prevent the occurrence of endotoxins within the G-CSF manufacturing process. G-CSF is a protein (biologic macromolecule with approx. 18.8 kDa) which can only be produced biotechnologically using recombinant *E. coli* bacteria. The specific bacterial cell line is genetically modified to over-express the recombinant protein as part of its metabolism. Besides the target protein, the host cells naturally produce a large number of other proteins and cell-specific substances (e.g. DNA molecules, proteins, bacterial endotoxins). These metabolic by-products cannot be prevented by means such as using other cell lines. Even if another (non-bacterial) cell line would theoretically be suitable to express G-CSF, the problem of having cell-specific contaminations within the G-CSF manufacturing process would be identical due to the biologic nature of a cell. Every cell is a living organism having a distinct and complex metabolism which has to produce a huge variety of different substances to ensure cell life and proliferation. For example, mammalian cells do not have any endotoxins (and may produce G-CSF in soluble form), but they glycosylate the protein. This glycosylation represents a major quality difference with the originator product, as it may also affect the specific bioactivity and/or immunogenic profile of the biosimilar product. Therefore, to make a biosimilar of Neulasta (non-glycosylated), biotechnological production using *E. coli* bacteria remains the best solution.

Besides the above-mentioned reasons, the establishment of a cell line for the production of a specific biologic molecule which is transferred into a drug substance for human use is generally a very complex R&D project on its own. The R&D is subject to immense laboratory work and regulatory obligations[3] before even starting the R&D on the actual manufacturing process of the target substance as e.g. described for G-CSF in the present AoA/SEA in chapter 3.7.3. Taking this into consideration and regardless of the non-applicability for avoiding Triton X-100 in G-CSF manufacturing, a completely different and much longer R&D timeline compared with the one presented in the AoA/SEA in chapter 4.5. would result.

Please note that it is technically not possible to produce large biomolecules (biosimilars) having molecular weights of 15.000 Da via simple multi-step chemical synthesis, in contrast to small molecule generics or peptides (approx. 150 Da). For illustration of the key difference between biosimilars and generics please refer to Table 1 below.

Table 1: Overview on key differences between biosimilars and generics

Properties	Small molecules generics	Biosimilars
Size (molecular weight)	small (approx. 150 Da)	large (approx. 150.000 Da)
Chemical structure	simple & well-defined	complex with potential structural variations
Complexity	easy to fully characterize	difficult to characterize
Storage & handling stability	relatively stable	sensitive
Potential adverse immune hazard	low	high
Manufacturing	predictable chemical process to make identical copy	specialized biological process to make similar copy
Manufacturing quality test	≤ 50	≥ 250

[3] ICH Topic Q 5 D Quality of Biotechnological Products: *Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products* (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-5-d-derivation-characterisation-cell-substrates-used-production-biotechnological/biological-products-step-5_en.pdf)

Approval requirements	small clinical trials in healthy volunteers (pharmacokinetic studies <50 subjects)	large clinical trials in patients (>300 subjects in total)

Definition Biopharmaceuticals

Biopharmaceuticals are drug products which are manufactured in, extracted from or semi-synthesized from biological sources like bacteria, plants, fungi but also humans. They can be composed of sugars, proteins or nucleic acids or complex combinations of these substances. Due to their origin from biological sources, the biopharmaceutical itself and also the respective biotechnological production process are subject to stringent regulations concerning product safety. A distinct hazard of biopharmaceuticals arises from immunologic or generally adverse reactions of the human body triggered by immunogenic or toxic impurities (e.g. proteins, DNA molecules, residual raw materials, etc.) possibly present in the final drug.

2. Regarding the PEGylation process, why has the minimum quantity been set to the amount it has? This prevents the use of potential alternatives, since it is a key parameter in your assessment. Is it not possible to perform the process in continuous flow?

The manufacturing process of PEG-Filgrastim can be divided in three main processes performed in separate equipment: (1) *Extraction and purification of G-CSF inclusion bodies,* (2) *Purification of G-CSF protein* and (3) *PEGylation of G-CSF protein.* Please refer to Figure 5 for an overview on the PEG-Filgrastim manufacturing process.

Figure 5: Overview on PEG-Filgrastim manufacturing

As described in chapter 3.7.3 of the AoA/SEA, the *extraction and purification of G-CSF inclusion bodies* requires eight successive washes using different buffer solutions (see Figure 6 of AoA/SEA) which are performed within a [redacted]. As a result of this sub-process, purified G-CSF inclusion bodies are obtained which need to be further processed and purified to "single" G-CSF proteins [redacted]. The minimum quantity of G-CSF inclusion bodies which can be used as starting material for the purification process of G-CSF protein is [redacted] (Note: yield of purified G-CSF inclusion bodies is directly linked to Triton X-100 usage and therefore set as key parameter for alternative assessment). Taking a certain percentage (approx. [redacted] of yield loss into account for the

G-CSF protein re-folding and purification process, the minimum quantity of [redacted] is required to ensure that sufficient purified G-CSF protein is obtained which can be used to feed the PEGylation process. The PEGylation process (resulting in PEG-Filgrastim) is tailored to handle a specific quantity of material from a process scale perspective (e.g. [redacted], etc.), i.e. the process was validated for a defined production yield and product quality. If a PEGylation batch process would need to be adjusted to lower amounts of G-CSF protein starting material, process validation would be lost and the impact on production yield and most importantly quality of product cannot be controlled. Evidently, the manufacturing process of PEG-Filgrastim has to be robust with consistent yields and quality of product to ensure paramount patient safety.

Concluding, yield losses during the extraction and purification of G-CSF inclusion bodies are critical, as they could potentially lead to the loss of a whole production lot which in turn leads to significant economic damage and a potential disruption of the product supply chain to other sites involved in the manufacturing of PEG-Filgrastim.

3. Please provide an update on the R&D performed since the application was submitted.

The first phase of the R&D experimental work "evaluation of Triton X-100 alternatives" has been started. As described in chapter 4.5.1 of the AoA/SEA, the alternative substance(s) (groups) mentioned in Table 9 and additional ones were evaluated on paper by comparison of (historical) literature, use profiles and GMP supplier assurance. As a result, three alternative substances (see Table 2 below) were assessed suitable for the subsequent experimental investigations [redacted].

Table 2: Alternatives selected for experimental investigations

Alternative substance (CAS)	Description of known properties
[redacted]	Non-ionic surfactant
[redacted]	Zwitterionic surfactant
[redacted]	Non-ionic surfactant

[redacted] experiments have been performed in which the G-CSF inclusion body (IB) washing process was performed using current process parameters but substituting Triton X-100 in the washing buffer solution ([redacted] by the respective potential alternative substance. Please refer to chapter 3.7.3.1 of the AoA/SEA for a detailed description of the purification process from a chemical perspective. A fourth alternative using just the [redacted] base buffer without Triton X-100 was also tested. For comparison of the effectiveness of the alternatives a control with Triton X-100 was included.

The liquid fractions (= wash supernatants) of the IB washes were collected and will be assayed for residual Host Cell Contaminants (Host Cell Proteins, Host Cell DNA and Bacterial Endotoxin) content in order to determine the relative clearance of these contaminants by the alternatives to Triton X-100. In addition, the purified IB pellets will be subjected to further processing to determine the residual Host Cell Contaminant (HCP) levels in the process stream that contains the product ([redacted]). At current stage, the results of the sample analyses are not yet available. However, if the performance of one of these alternatives is considered sufficient [redacted] required for an optimum clearance of residual HCPs and maximum yield of G-CSF target product. Please refer to chapter 3.7.3.2 of the AoA/SEA for a detailed description of the purification process from a systemic perspective.

[redacted]

[redacted]

4. Regarding the paper-based comparisons, this step seems easier to perform than the others. Why has this not been done already before?

As described in the AoA/SEA in chapter 4, "the first phase of the technology transfer of the G-CSF manufacturing process from Merck (Martillac) to Fujifilm (Billingham) is the establishment of the current Triton X-100-based process [redacted] The purpose is to ensure that the production process can be replicated at Fujifilm, meeting all critical requirements as per the current process control strategy. These requirements include foremost process yield and product quality specifications during the process and for the intermediate G-CSF. Following successful establishment of the process at laboratory scale the evaluation of Triton X-100 replacements will take place."

Since both parties involved, Merck Martillac and Fujifilm, are fully occupied with the obligations to ensure the targeted market launch of PEG-Filgrastim in [redacted] with the Triton X-100-based manufacturing process in combination with the Technology Transfer in parallel, the exhaustive paper-based evaluation of potential alternatives could not be performed with a meaningful and satisfactory result, i.e. selecting the most promising substances for experimental investigation from the substance groups mentioned in Table 9 in chapter 4.4.1. of AoA/SEA. Additionally, the detailed paper-based analysis of potential alternatives would not speed up the R&D process for alternative identification since the time limiting steps are the necessary [redacted] investigations for each of those substances. Please refer to the response to Q.3 for further information on the current R&D status of these investigations.

Concluding, even if the paper-based evaluation would have been conducted earlier, the *Development & Substitution process and Technology Transfer* plan described in chapter 4.5. would remain the same, i.e. at first transfer of the Triton X-100-based manufacturing process from Merck (Martillac) to Fujifilm (Billingham) and after establishment [redacted] start with [redacted] investigations on potential alternatives. In other words, the required review period of <u>at least nine years</u> would remain the same.

For further information please also refer to chapter 4.1.1. of the AoA/SEA.

5. You mention that other biosimilars for Neulasta have already been approved in the EU. Do you know if these products also rely on OPE/NPE?

In general, the manufacturing process of any pharmaceutical substance, i.e. biopharmaceuticals[5] or synthetic pharmaceuticals, is strictly confidential and business critical company information. Therefore, the applicants and the company Fresenius do not know about the manufacturing processes of competitor products and are thus clearly not aware if these rely on OPE/NPE related substances.

Details on the manufacturing process of the originator product Neulasta were not disclosed after the patent has expired.

[5] Biopharmaceuticals are drug products which are manufactured in, extracted from or semi-synthesized from biological sources like bacteria, plants, fungi but also humans. They can be composed of sugars, proteins or nucleic acids or complex combinations of these substances.

SEAC questions about the Socio-Economic Analysis:

1. **Please provide more information on the sales and profit margin assumptions. What are they based on? Can you also provide a non-confidential range for the profit margin?**

As presented in the AoA/SEA document, the product is planned to be launched in XXXX. [redacted] below shows the forecast for sales revenues in millions of Euros from [redacted] on.

[redacted]

Forecasted sales are based on various assumptions:

- Overall market penetration of biosimilars products *vs.* originator: depending on the country and the year considered, an assumption of [redacted] is made.
- Market share gained vs. other biosimilar competitors: this depends on the order of entry of the drug on a given market (launch date), the sales force and marketing strategy put in place, etc. Depending on the country and the year considered, an assumption of [redacted] market share is made.
- Price erosion: biosimilars price discounts *vs.* originator list price pre-biosimilars entry is anticipated to be within the following range at peak: [redacted] (depending on competition intensity and market archetype)

In terms of profitability, it is estimated that PEG-Filgrastim could reach an annual ratio of after-tax profits to net sales of [redacted] as production scales up. Profit margin is calculated by subtracting the following costs from revenues: production costs, [redacted], logistical and administrative costs, marketing & sales investments, taxes. The applicants are not prepared to share any non-onfidential values about the profit margin.

2. **Please clarify which entity would suffer the profits loss described in section 5.1.2. Is this Fresenius Kabi SwissBioSim GmbH or Fresenius Kabi Germany GmbH?**

   Fresenius Kabi SwissBioSim GmbH is a fully-owned subsidiary of Fresenius Kabi Deustchland GmbH. Fresenius Kabi SwissBioSim GmbH is an entity dedicated to the development and approval of biosimilars, and as such is not selling the biosimilar products to third parties, as the commercialization is made at the level of local entities on a country basis. All the profits made from the commercialization of biosimilar products will eventually be consolidated at the level of Fresenius Kabi Deustchland GmbH and Fresenius Kabi SwissBioSim GmbH [redacted].

3. **Regarding a potential effect on the market from the interruption of supply of PEG-Filgrastim by the applicants under the NUS:**

   a. **Would competitors be able to supply the PEG-Filgrastim that would have been supplied by the applicants under the Applied for Use scenario? If so, is it possible to predict if these competitors would be producing within or outside the EEA?**

   As stated in the SEA, it is anticipated that about 5-6 biosimilars of PEG-Filgrastim might be approved by 2021 in each region; based on today's approvals and dossier reviews by the US agency (FDA), this number of competitors might rather be between 3-4 for the US market.

   It is difficult to anticipate the full production chain for all these potential competitors but the drug substance production sites, as reported in publicly available assessments from authorities, seem to be in great majority located outside the EEA: Bangalore (India), Ahmedabad (India), Spain, Boulder (USA), Slovenia, Navi Mumbai (India).

   Assuming all those competitors would be able to launch in the EU or other regions, they could likely supply the market demand in the absence of Fresenius Kabi compound, possibly after some months to adjust their production capacity. However, the reduction of competition (due to a non-granted authorisation) would have an impact on the prices, so it could potentially reduce the access to the drug for some patients (see answer to next question).

   b. **Would you expect there to be any impacts on patients, whether on their health, or on the affordability of their treatment?**

   Yes, an impact would be expected as a non-granted authorisation would induce a **lower competition**, so an overall lesser effect on price reduction: fewer patients could potentially benefit from this treatment. In addition, Fresenius Kabi is intending to supply differentiated solutions/presentations to the market, aiming at improving the patient's experience with this treatment and at optimizing the support provided to the patients.

<u>SEAC question on distributional impacts</u>:

9.The current opinion template includes an overview table on distributional impacts (see below). Please complete the table below by adapting it to your circumstances (note that not all entries may be relevant for your case), as it will be helpful when addressing distributional impacts in the opinion.

Note 1: for economic impacts "+" indicates gain and "-" indicates loss

Note 2: for environmental impacts "+" indicates increase in environmental impacts while "-" indicates reduction

Note 3 → Severity of impacts: using scale high (+++ or ---), medium (++ or --), low (+ or -) or not applicable (n/a)

Affected group[1]	**Economic impact**	**Environmental impact**
Economic operator		
Applicants (CMOs)	**- -**	**No change**
Product owner (Fresenius Kabi)	**- - -**	n/a
Competitors in the EU (manufacturers of other biosimilars or originator products)	**+++** (in case they don't use OPE or have an authorisation)	**+** (assuming that emissions can only be increased in comparison to the situation described in the present application)
Competitors outside the EU (manufacturers of other biosimilars or originator products)	**+++** (most competitors are believed to produce outside EEA)	**+++** (assuming regulations outside are less strict concerning the use of OPE)
Patients requiring oncology treatment	**- -**	n/a
Geographical scope		
EU	**- - -**	**No change**
Non-EU	**+++**	**+++**
Within the applicants' or related parties business (CMOs or product owner)		
Employers/Owners	**- - -**	n/a
Non-exposed workers and other employees	**- - -** (as a result of dismissals)	n/a