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GMP and occupational safety requirements before during and after the lyophilisation process

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ABSTRACT

The article provides a detailed overview about possible operator and patient exposure to highly potent API (HPAPI) from potential contamination and cross contamination through the lyophilization process and supporting technologies like Isolators. The aim of this article to raise awareness of the risk to the operators and the patients during loading and unloading a lyophiliser and the lyophilizing process itself. Specific consideration is taken of cleaning limits for nonproduct contact surfaces in the isolator and of the interior of the Lyophiliser which are define as non-direct or indirect product contacts parts. Furthermore, various technical measures are described of the Isolator System and the Lyohiliser to prevent the contamination of the operators with the highly active substance or the risk of cross contamination between two different products produced after the other.

INTRODUCTION

The number of highly active or highly dangerous substances has increased significantly in recent years. Global estimates indicate that there are more than 1000 new pharmaceutical products under development that are classified as highly active. Many of these new products are small molecule or bio-pharmaceutical products, or a combination of both referred to as ADCs – "Antibody Drug Conjugates". ATMPs, "Advanced Therapy Medicinal Products", such as gene and cell therapies are often highly active products as well and require a high degree of operator and product protection.
In order to meet the respective requirements, process systems such as the freeze drying process and associated processes like loading and unloading of the freeze dryer should be designed for highly active substances. The issue of cleaning to avoid cross-contamination in multi-purpose systems is also gaining in importance with the introduction of PDE "Permitted Daily Exposure" by the EMA "European Medicines Agency" in 2014.

Regulatory GMP and occupational safety requirements for highly potent substances

Product protection and occupational health and safety must be reliably observed in pharmaceutical production. A risk analysis that takes both of these aspects into account must be carried out in order to reconcile these protection goals, which are often perceived as contradictory.

Toxicological data serves as a basis for cleaning and exposure limits for work with any substance. The scientific toxicological approach is identical for the permitted daily exposure limit (PDE) and for the occupational exposure limits (OEL). It should be noted, however, that very different routes of exposure are defined for PDEs (in the case of lyo products usually the parenteral route), whereas the OEL only considers inhalation exposure.

Exposure measurements such as air measurements in production environments and results of swab tests on surfaces can be used as an argument for controlling cross-contamination by air and mechanical means (1).

The regulatory requirements regarding GMP and cross-contamination in Europe are largely regulated in EU GMP Part 1 for medicinal products and Part 2 for active substances. Regarding the requirements for preventing cross-contamination, there have been significant changes since 2014. In Part 1, Chapters 3 (premises and equipment and their potential for reducing cross-contamination) and 5 (production) are particularly relevant to the containment discussion, with Annex 1 also being relevant for aseptic production. This annex is currently under revision, but the published draft already has a certain relevance.
How these GMP-relevant limit values are to be determined toxicologically is specified in detail in the guideline *"on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities*".

In this context, the PDA publication "Preventing Cross-Contamination during Lyophilization" should also be noted, where, from a GMP and EHS point of view, "safe surface limits" have been assessed in a very conservative but helpful first approach. This assessment can be used both for isolators and for the lyophiliser itself. Particularly with regard to cross-contamination, however, it can be assumed that it is possible to adapt the limit values to the respective application by means of measurements.

This assessment is based on a palm of the hand concept. With regard to occupational health and safety, it is frequently assumed that trained employees in an area where increased contamination is expected will not pick up more than 1 dm^2 of deposit per day through skin contact. In an area where no more contamination is expected, e.g. in a " clean corridor ", it is assumed that less caution is exercised and 10 hand contacts per working day might occur. Therefore, a maximum of one tenth of a PDE may be present on a 1- dm^2 surface. This would result in maximum exposure to 1 PDE per shift.

Table 1 indicates that visual cleanliness is often not sufficient for highly active substances. An initial value of approx. 4 µg/cm^2 is often given for visual cleanliness, but this should be verified by measurements. Since visually clean is necessary but not sufficient, swab tests are recommended after cleaning.

Simplified assumption OEL= PDE / 10 To be verified by expert team and based on tox data		Limit for surface with no direct or indirect product contact inside the Lyophilizer (µg/dm²) GMP and Operator safety	Limit for "public" surface with uncontrolled possibility of unprotected hand contact (µg/dm²) Driven by operator safety
PDE (µg/d)	OEL (µg/m³)		
10000	1000	Visually clean	Visually clean
1000	100	Visually clean	100
100	10	100	10
10	1	10	1
1	0.1	1	0.1
0,1	0,01	0,1 or lower	0,01 or lower

Table 1. Cleaning limit values for freeze dryers and environment.

In addition, contamination outside of the containment should also be considered in the risk analysis. When working in isolators, substance leakage is usually greatly reduced. Often, however, the containment is not an isolator, so that employee protection must be ensured in such cases as well.

Maintaining air concentrations below the OELs and low-contamination surfaces that can also be verified by swab tests help to prevent GMP-relevant cross-contamination issues.

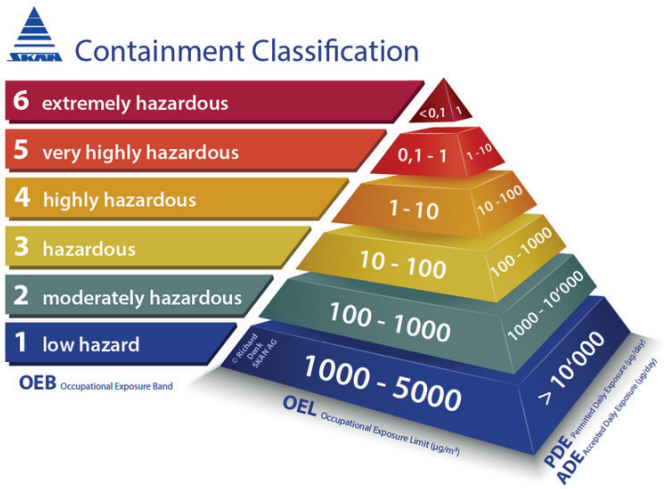

Figure 1. Containment classification. Conversion from PDE/ADE to OEL is shown in simplified form.

Barrier Solution for loading and unloading the lyophyliser

Loading of a freeze dryer takes place either in a classic clean room constellation with Grade A protection zone and Grade B background or by means of a barrier system such as an RABS (Restricted Access Barrier System), or an isolator. In the case of an RABS, the same general conditions apply as for a conventional cleanroom constellation, i.e. zone A is located inside the RABS and zone B is outside. The RABS system offers an additional advantage in that the operators are isolated from the product by a barrier such as closed transparent doors that may have glove ports. The RABS system is cleaned and disinfected together with the clean room. However, neither the conventional solution nor a RABS system is suitable for highly potent substances, as they do not provide any protection for production personnel but are designed for pure product protection. In this respect, isolators have a significant advantage, as they offer a continuous barrier system as well as zone separation between the sterile area inside the isolator of zone A and zones C or D outside of the isolator. The isolator also has an integrated vaporized H_2O_2 decontamination system that maintains the aseptic zone by means of a validated cycle development, from leakage testing of the isolator, conditioning of humidity and temperature to subsequent decontamination and aeration of the vaporized H_2O_2 until the required residual limit value is reached. See figure 2.

Figure 2. Isolator for loading and unloading a freeze dryer.

Isolator technology meets the GMP requirements for the production of sterile products and it is also the preferred technology in terms of national and international regulatory requirements. In order to make the isolator suitable for handling highly active substances, some additional technical protection measures are required.
The following information and technical measures should be observed:

- During lyophilisation, a phase transition from solid to gas takes place to separate the solid substance from the solvent/water and isolate the freeze-dried pharmaceutical product. During this process, external contamination of adjacent primary containers such as the vials may occur. In addition, during lyophilisation and final insertion of the lyo stopper into the vial, the vial itself may be damaged or break, which results in additional contamination when the lyophiliser is unloaded.
- For GMP reasons, the isolator adjusted on the lyophiliser is operated at overpressure to the surrounding room to prevent microbiological and particulate contamination from outside. This means, however, that any highly active substances that are released can escape to the outside if the isolator leaks.
- If the isolator line has a validated stopper height detection prior to crimping the vials, the isolator can also be operated under negative pressure during unloading. Any stoppers for which the validated stopper height has been exceeded may not be crimped and are rejected beforehand.
  Operating the isolator under negative pressure creates an additional safety factor for the operating environment.
- Another protective factor is to avoid any installations in the Isolator zone below the critical unloading zone on the lyophiliser. Any feed-throughs from inside the isolator through the isolator wall such as cable throughputs, lifting devices, sensors or transfer ports like RTPs (Rapid Transfer Ports) pose a risk of contamination of the highly active substance from inside the isolator to the outside.
- To maintain clean room class A inside the isolator, the conditioned air is fed unidirectionally through HEPA 14 filters into the aseptic isolator chamber at an air speed of 0.45m/sec (+/-20%). The air is recirculated to save energy costs. Due to this recirculation, product particles are carried into areas that are difficult or impossible to clean. To prevent this, filter cartridges like FiPa are installed in front of the recirculation ducts. They prevent the highly active substance from spreading and safely collect it in the filters. The filter cartridges have a safe filter change feature and prevent recontamination into the isolator

chamber as well as contamination towards the operator during filter change.

- It is also important to look at the hygienic design of the systems built into the isolator, such as the lyophiliser loading and unloading system. Vials that have been contaminated or broken by the lyophilisation process will also contaminate the loading and unloading system when the vials are unloaded. These units and the entire interior of the isolator are cleaned after each batch. Cleaning requirements and their limit values were published by the PDA (Parenteral Drug Association) (2). See Table 2, isolator cleaning limit values.

Simplified assumption OEL= PDE / 10 To be verified by expert team and based on tox data		Limit for surface with no direct product contact inside the isolator ($\mu g/dm^2$) GMP and Operator safety	Limit for "public" surface with uncontrolled possibility of unprotected hand contact ($\mu g/dm^2$) Driven by Operator safety	Limit for airborne API inside of isolator after cleaning at product changeover ($\mu g/dm^2$) Driven by GMP
PDE ($\mu g/d$)	OEL ($\mu g/m^3$)			
10000	1000	Visually clean	Visually clean	10000
1000	100	Visually clean	100	1000
100	10	100	10	100
10	1	10	1	10
1	0,1	1	0,1	1
0,1	0,01	0,1 or lower	0,01 or lower	0,1 or lower

Table 2. isolator and environment cleaning limit values.

Compliance with all these safety precautions can result in very low limit values (OELs). The entire process should be evaluated in advance in a risk assessment and the necessary safety measures defined.

LYOPHYLISING REQUIREMENTS FOR PROCESSING HIGHLY ACTIVE SUBSTANCES

The interface between the filling line with the isolator and the freeze dryer is formed by the loading and unloading system, which usually pushes rows of vials onto the freeze dryer's trays during loading. After freeze-drying is completed, the system then transports the vials back out of the freeze-dryer.

Key parts of the loading and unloading system are in the isolator in front of the freeze dryer. The same requirements apply to the installed assemblies of the loading and unloading system as well as all other assemblies of the filling line which are located in the isolator.
It should be noted, however, that some components are moved both in the isolator in front of the freeze dryer as well as in the freeze dryer, while there is no laminar airflow in the freeze dryer due to the design.
In order to reduce potential hazards, the following aspects should be considered for the design of the loading and unloading system:

- Fully automatic operation of the system
- Ideally no movement above the vials
- Robust design with few mechanical components
- All surfaces can be cleaned
- H_2O_2 "decontamination" of all surfaces
- Accessibility of all elements during qualification

Especially with highly active substances, the freeze dryer itself is also subject to special requirements. The product chamber, the ice condenser and all pipes, e.g. the vacuum system, must be classified as indirectly in contact with the product. It cannot be ruled out that particles may be carried into a vial during freeze-drying. This can be caused, for example, by the water vapour flow during freeze-drying, turbulence during ventilation before closing the vials or by vial breakage.

During the increasingly common process step of controlled nucleation for simultaneous freezing of the product in all vials, particles may also be transported into the vials.
The freeze dryer can be cleaned and sterilized by means of the relevant fully automatic CIP/SIP processes, and the loading and unloading system can be cleaned and decontaminated. However, in many cases, manual intervention by an operator remains necessary, sometimes being complex and time-consuming. In recent years, there have been some promising developments in this area, which significantly reduce such effort.

Figure 3. Advanced loading and unloading system for a GMP-freeze dryer.

In principle, a transmission of particles between the freeze dryer and the isolator is possible, therefore both subsystems need to be perfectly matched to each other. Complete systems with a compact design, in which the product chamber and the ice condenser of the freeze dryer are combined, offer advantages in this regard. Benefits include the small footprint as well as easy accessibility of all components.

After freeze-drying, the product is stored as a lyo-cake in the vials. It must generally be assumed that product particles are present in the freeze dryer, the loading and unloading system and on the outside of the vials. These particles can be carried into the isolator as well as into the capper station, for example. Since the lyo-cake are solid particles, operator protection is particularly important for highly active substances. For this reason, isolator systems with external cleaning of the crimped vials before leaving the isolator are useful.

Especially when dealing with highly active substances, special attention must be paid to the vacuum system. Despite causing a loss of pressure, it is recommended to use filter systems in the pipeline leading to the vacuum pump in order to protect the vacuum pump, the entire exhaust system and the "outside room" from product particles. The filter system should be designed as a redundant bag-in/bag-out system in order to be able to replace the filters safely and regularly. The filters are usually to be placed in the technical area close to the product chamber of the freeze dryer. The vacuum pipeline leading to the filter must be equipped with appropriate CIP/SIP devices.

Waste water and any water/solvent thawed after freeze-drying is complete should be deactivated using suitable systems, e.g. a kill tank. A switchable discharge on the freeze dryer is recommended for this purpose.

If different substances are handled consecutively during freeze-drying, the interior of the freeze dryer should be cleaned in accordance with the toxicity of the active ingredient to prevent possible cross-contamination with

the subsequently manufactured pharmaceutical product. A group of experts has published a PDA (Parenteral Drug Association) publication on this topic in 2019.(3) See table 1

Apart from normal operation of the system consisting of freeze dryer and the loading and unloading system, emergency situations must also be considered. To this end, appropriate SOPs must be developed within the framework of risk assessments in order to permit continued production of a high quality of the pharmaceutical product on the one hand and to protect the operator on the other.

CONCLUSIONS

Highly active or highly dangerous substances can be made safe for the loading and unloading process of the freeze dryer by technical measures such as the use of isolators. Technical measures should also be implemented in freeze-drying to prevent the spread and transmission of highly active substances. Cleaning requirements in accordance with the pharmaceutically effective requirements apply to product protection in a multi-purpose system as well as to operator protection when opening the freeze dryer or isolator for modification, maintenance or inspection tasks. In any case, a comprehensive risk assessment should be carried out by the user during design execution in order to determine the GMP aspects of cross-contamination as well as the EH&S "Environment Health & Safety" requirements and to implement them by means of technical measures.

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