Sanofi Holistic Risk-Based Approach to Low Endotoxin Recovery (LER)

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Sanofi and Sanofi Pasteur

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Jon Williams has worked in Quality Risk Management (QRM) for the Sanofi Biologics organization for the past 7 years where he supported manufacturing sites in the implementation and integration of QRM principles throughout the site quality organization including training on formal risk tools (FMEA, HACCP, etc.) and the development of custom risk tools. Jon's current role is with the Sanofi Global Proactive QRM Program tasked with implementing a proactive QRM Program across the entire Sanofi organization by providing harmonized practices targeted at anticipating and avoiding unacceptable risks based on ICHQ9 (Risk Management) guidelines.

Introduction

Low Endotoxin Recovery (LER) has been THE hot industry topic for many years now and still there are debates on some fundamental points. Fortunately, the collective efforts of regulatory authorities, biopharma manufacturers, industry groups, as well as instrument and reagent vendors have provided a much clearer direction over the last five years. This paper describes the Sanofi holistic approach to LER, which was generated by an internal group of risk management and endotoxin subject matter experts. The harmonized approach leverages the existing knowledge base and closes the gap in some key areas with a more comprehensive toolkit than previously described in the literature.

This paper also intentionally avoids some of the ongoing debates regarding the "true mechanism" of LER, appropriate uses of Reference Standard Endotoxin/Control Standard Endotoxin RSE/CSE vs. Naturally Occurring Endotoxin (NOE), and potential medical or patient impact. Those topics are well documented in previous work and ongoing work from other authors and industry sub-teams.

The focus of this paper is a description of the practical toolkit to address LER for investigational medicinal products and previously registered commercial products. The design of the commercial product risk-based approach is described along with a summary of the use of the Sanofi Endotoxin Ingress Risk Assessment (EIRA). The study-based investigational product approach is described along with an investigational product LER case study.

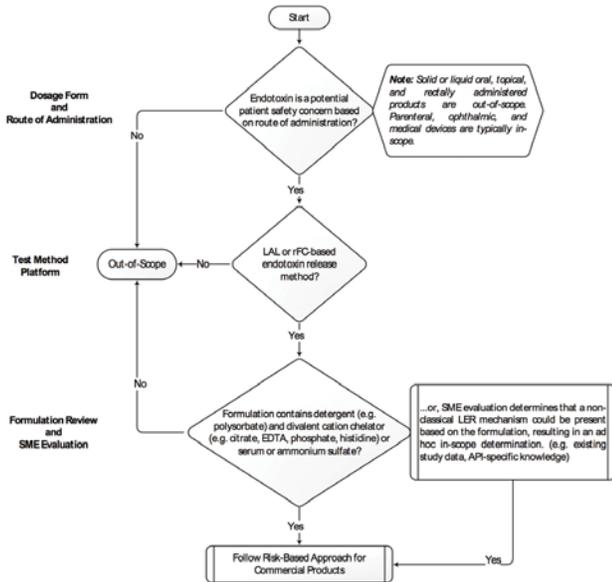## Risk-Based Approach for Registered Commercial Products

The principles of quality risk management as defined in ICH Q9, *Quality Risk Management*, were leveraged in the generation of the commercial product strategy. The approach was designed to dovetail with any existing microbial control strategies and supporting risk assessments. The Risk Assessment facilitates information-based decision mechanisms by evaluation of the current product and process-relevant risks, existing control effectiveness, and any pharmacovigilance field signals. It was designed as a beneficial enhancement to mature proactive risk programs or as a stand-alone approach for developing risk programs.

Scope Determination

In order to determine which legacy commercial products are in-scope for deeper evaluation of LER four key elements are considered:

- Product type, route-of-administration, or potential endotoxin safety concern/testing
- Formulation components in the drug product
- Test method reagents/platform
- SME evaluation or other known factors that affect scope determination (e.g. possible non-classical LER mechanisms - masking)
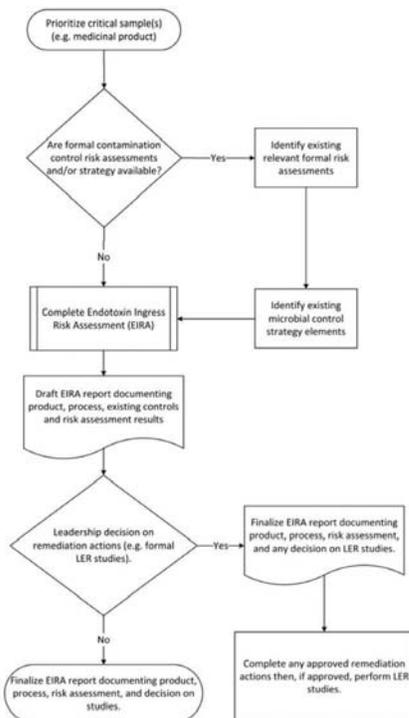
An example flow chart for the scope determination is provided below:

Risk-Based Approach Overview

The Sanofi commercial products approach is a multi-step process that focuses on a customized risk assessment format developed specifically for LER called the Endotoxin Ingress Risk Assessment (EIRA). The EIRA was designed to assess the overall endotoxin ingress risk for in-scope product samples. Although the EIRA provides a mechanism to reach a final rating for the overall risk of endotoxin contamination, it should be supported by a technical report that documents supporting data, rationale, and justification for the ratings.

An example flow chart for the risk-based approach is provided below.

EIRA Overview

The EIRA is a structured evaluation of current potential risks, effectiveness of existing controls, and product field data. The EIRA team may include representatives from departments such as:

- Microbiology
- Manufacturing
- Risk Management
- Facilities
- Utilities
- Regulatory
- Quality Management
- Contamination Control
- Pharmacovigilance

The EIRA team rates the individual endotoxin ingress risk elements (numerical) and individual endotoxin ingress control elements (numerical) which are then compiled to arrive at a preliminary risk level (low, medium, high). Available product field data is then considered to arrive at an overall endotoxin ingress risk level (low, medium, high). The overall endotoxin ingress risk level is then compared to an action table for proposed mitigation activities.

A diagram of the EIRA design components is provided below:

The individual endotoxin ingress **Risk Elements** included in the assessment are:

- Hygiene Program
- Employee Training and Aseptic Technique
- Manufacturing Process – Open Processing and Room Classification
- Manufacturing Process – Clean (and/or sterilized)/Dirty Equipment
- Manufacturing Process Design
- Raw Materials and Components Testing
- Facility Design – Standing Water
- Facility Sanitization Disinfectant Efficacy
- Facility Design – Area Classification
- Facility Design - Maintenance and Facility Conditions
- Equipment and Components (Endotoxin Levels and Validation)
- Utilities – Water, Steam, Gas
- Utilities – Rouge and Passivation

The individual endotoxin ingress **Control Elements** included in the assessment are:

- Personnel – Hygiene, Training and Gowning
- Historical Product Stream Gram Negative Bioburden Results

- Raw Material and Components – Historical Performance
- Raw Material and Components – Supplier Performance
- Utilities Requalification Performance
- Environmental Monitoring Results
- Environmental Monitoring Performance

The **Field Data** included in the assessment is:

- Pharmacovigilance Review

EIRA Execution Process, Details, and Examples

Step 1 - Rate the individual endotoxin ingress risk elements.

An example of the individual risk element rating for manufacturing process (open processing and room classification from the EIRA is provided below – lower scores represent lower risk):

	Rating	Criteria	Score	Score Result
Manufacturing Process Open Processing and Room Classification	**Low**	Manufacturing performed in a fully closed system or within barrier isolators or restricted access barriers (RABS) for open process steps.	1	
	Medium	Manufacturing performed in appropriately classified area required per regulation for the operation, but without the use of barrier isolators or restricted access barriers (RABS) for open process steps.	3	
	High	Manufacturing not performed in appropriately classified area required per regulation for the operation without the use of barrier isolators or restricted access barriers (RABS) for open process steps.	5	

Step 2 - Determine the Endotoxin Ingress Risk Level

Once all the other individual endotoxin risk elements (Hygiene Program, Employee Training and Aseptic Technique, etc.) are rated using their own corresponding scoring scale, the total is calculated and compared to a pre-defined scale, similar to the example in Table 1 below:

Table 1. Potential for Endotoxin Contamination	
Endotoxin Ingress Risk Levels	**Sum of the Risk Element Score Ranges**
High	> 46
Medium	23 – 46
Low	< 23
Sum of Risk Elements:	**Total:** _____

Step 3 - Rate Individual Endotoxin Ingress Control Elements

An example of the effectiveness of controls individual element rating for historical process stream Gram negative rod bioburden results is provided below. Lower scores correspond to higher ratings, representing more effective control performance:

	Rating	Criteria	Score	Score Result
Historical Process Stream Gram Negative Rod Bioburden Results	**High**	Over the past 12 months, bioburden results related to Gram negative rods for process stream samples were consistently within specifications or limits with no adverse trends identified.	1	
	Medium	Over the past 12 months, bioburden results related to Gram negative rods for process stream samples had isolated instances of Out-of-Specification (OOS), Action Limit excursions, or Out-of Trend (OOT) results.	3	
	Low	Over the past 12 months, bioburden results related to Gram negative rods for process stream samples have failed specification or exhibited multiple instances of OOS, Action Limit Excursions, or adverse trends.	5	

Step 4 - Determine the Controls Effectiveness Level

Similar to the risk elements, after each of the other individual control effectiveness elements are rated using their own corresponding scoring scale. The sum of the ratings is calculated and compared to a pre-defined scale, similar to the example below in Table 2 below:

Table 2. Effectiveness of Controls	
Controls Effectiveness Level	**Sum of the Control Element Score Ranges**
Low	> 26
Medium	14 – 25
High	< 14
Sum of Effectiveness of Controls:	**Total:** _____

Step 5 - Determine the Preliminary Risk Level

The preliminary risk level is determined by using the Endotoxin Ingress Risk Level calculated from Table 1 and the Controls Effectiveness Level determined from Table 2. The intersection point of the levels on the grid in Table 3 below establishes the preliminary risk level.

Table 3. Preliminary Risk Level				
		Controls Effectiveness Level (from Table 2)		
		High	**Medium**	**Low**
Endotoxin Ingress Risk Level (from Table 1)	**High**	Medium	High	High
	Medium	Low	Medium	High
	Low	Low	Low	Medium

Step 6 – Determine Individual Rating for Pharmacovigilance Review of Field Data

To determine if there are any signals from field, 12 months of data is reviewed by pharmacovigilance. Determine the level (Confirmed or Not Confirmed) for using Table 4.

Table 4. Pharmacovigilance Review of Field Data Level			
Data Source	Level	Criteria	PV Review of Field Data Level
Pharmacovigilance Review	Confirmed	A review of the last 12 months of safety data from external sources (e.g. adverse events) and internal sources (e.g. Periodic Product Review) and summarized in the Periodic Benefit Risk Evaluation Report (PBRER) confirms no incidents related to pyrogenic or any other adverse reactions attributed to a suspected or proven endotoxin contamination that would indicate product safety concerns. The benefit/risk profile of the product indicates no latent pyrogenic activity in the product.	
	Not confirmed	A review of the last 12 months of safety data from external sources (e.g. adverse events) and internal sources (e.g. Periodic Product Review) and summarized in the Periodic Benefit Risk Evaluation Report (PBRER) could not confidently confirm that incidents related to pyrogenic or any other adverse reactions attributed to a suspected or proven endotoxin contamination have not occurred.	

Step 7 - Determine the EIRA Result

Using Table 5 determine the intersection of the Preliminary Risk Level from Table 3 and the individual rating for Pharmacovigilance Review of Field Data in Table 4. The intersection point is the EIRA Result (High, Medium, or Low). Record the EIRA Result in Table 5 below.

Table 5. EIRA Result			
		PV Review of Field Data Rating (from Table 4)	
		Confirmed	Not confirmed
Preliminary Risk Level (from Table 3)	High	Medium	High
	Medium	Low	Medium
	Low	Low	Medium
		EIRA Result:	

Step 8 - Determine the Recommended Risk Mitigation Actions

Using Table 6, identify recommended risk mitigation actions to reduce the risk to acceptable levels.

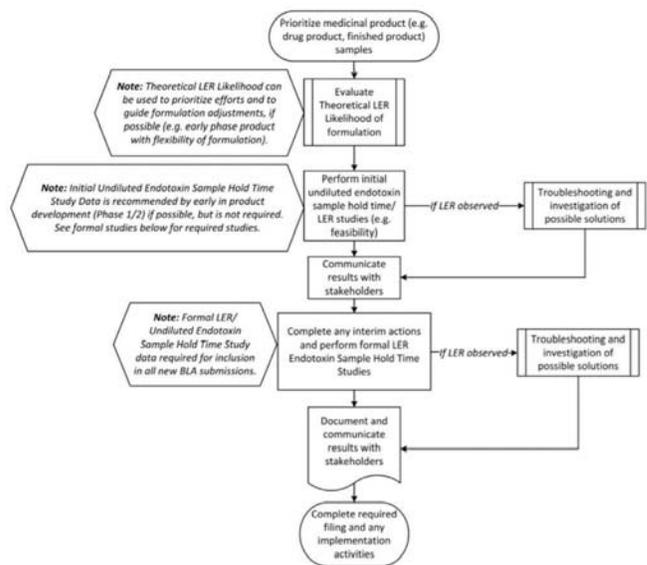| Table 6. Recommended Risk Mitigation Action Table	
EIRA Result (from Table 5)	Action Table
High	The risk associated with potential endotoxin contamination is not acceptable. Additional risk control measures are required to reduce risk to within an acceptable level. Further evaluation is required via a formal risk assessment tool (i.e. HACCP, FMEA) before an action plan can be developed.
Medium	The risk associated with potential endotoxin contamination may be acceptable provided additional measures are taken (e.g. risk control or mitigation measures, validation, etc.) or appropriate justification is documented. Further evaluation via a formal risk assessment tool (i.e. HACCP, FMEA) may aid in development of an action plan.
Low	The risk associated with the potential endotoxin contamination is acceptable. No mitigation is required.

The justification for each individual rating within the EIRA described above must be supported and documented in an EIRA report. The ratings alone are not considered sufficient. Related existing microbial control risk assessments (e.g. HACCP) should be cross-referenced, as should any supporting microbial control strategies or applicable procedures.

The EIRA outcomes, risk mitigation, and justification provides an evaluation of the overall risk of endotoxin ingress for a given process and product. That information can then be used for leadership to make an informed decision and prioritize the appropriate next actions (e.g. risk mitigation, formal risk assessments, formal LER hold time studies, no formal LER hold time studies.)

Strategy for Investigational Medicinal Products

The strategy for investigational medicinal products is similar to the previously registered commercial product, however formal LER studies are required for all new BLA filings. Therefore an EIRA is not required for these products since there is no assessment required to instruct the appropriate path forward.

The approach for investigational medicinal products is summarized in the flow chart below:

LER Theoretical Likelihood Tool Overview

Although no universally accepted mechanism for LER has been identified, some theoretical estimations can be made. Components that are known potential contributors to "classical LER" are well documented in the literature. For example, a surfactant (e.g. polysorbate) combined with a divalent metal ion chelator (e.g. citrate, EDTA). Some "non-classical LER" masking factors have also been reported (e.g. serum, ammonium sulphate, uncommon pH levels). Subject matter expert (SME) evaluation of other product-specific factors or existing studies may also improve the estimation of the theoretical likelihood of LER. LER likelihood can help prioritize activities or direct more focus to certain products. The estimation of likelihood does not replace the requirement of formal LER studies for new products, regardless of the result of the evaluation. The basic theoretical LER likelihood rating tool design based on FDA presentations of drug sponsor BLA results, external experience, and internal Sanofi experience is provided in this chart:

Theoretical Likelihood Rating	Criteria (or as justified in SME assessment comments)	Rationale for ratings based on literature sources
Very High	Surfactant (e.g. polysorbate) + citrate or EDTA, ammonium sulfate, or serum/albumin	Estimated at >30% of formulations of this type
High	Surfactant (e.g. polysorbate) + phosphate	Estimates at ~20 - 30% of formulations of this type
Medium	Surfactant (e.g. polysorbate) + histidine	Estimated at ~10 - 20% of formulations of this type
Low	Surfactant (e.g. polysorbate) + other chelator	LER unlikely, however some similar combinations reported occurrence of LER
Very Low	No surfactant and/or no chelator, or existing negative LER study data	LER chance is remote, no known or similar combinations reported LER

LER Endotoxin Sample Hold Time Studies

LER related activities and studies must be planned and performed at the appropriate clinical stage to ensure successful outcomes. Feasibility studies are recommended during early stages to allow time for troubleshooting and remediation prior to formal studies and to ensure safety of future clinical batches.

In Phase 1, recommendations are to avoid known high theoretical LER likelihood formulations when possible and consider LER feasibility studies especially for high likelihood LER formulations. For many product types it is challenging to avoid divalent metal ion chelators and surfactants, but when possible the judicious selection of excipients and concentration can reduce the theoretical likelihood of LER.

In Phase 2, if not already completed, LER feasibility or formal studies should be performed depending on product strategy and timing. If known, the routine operations test conditions should be matched and study designs should follow best practices required for formal studies.

In Phase 3, formal LER studies matching routine operations test conditions are required. Supporting Rabbit Pyrogen Test and/or Monocyte Activation Test data must also be generated as required per regulatory requirements.

Principle

During LER/HT testing, undiluted samples are spiked with RSE or CSE. Samples are then held for a length of time in a container and at a temperature relevant to that particular test article and manufacturing process. At time points throughout the holding time, the sample is then diluted to the working dilution determined for routine testing and tested for endotoxin. The readout at time T is the percent CSE or RSE spike recovery in the sample, compared to the percent spike recovery in the LAL reagent water (LRW) negative control at the same hold time T. LER is diagnosed and confirmed if the CSE/RSE spike recovery in the sample is <50% for two consecutive time points. The demonstrated stability of assayable endotoxin content over time is the latest time point tested that the percentage of CSE/RSE spike recovered is strictly greater that 50%.

Case Study

The procedure for LER studies described above was applied to an investigational vaccine currently in Phase 3.

The DP (drug product) stage was selected for this assessment as the Bacterial Endotoxin Testing (BET) is performed on this stage for release.

Theoretical Low Endotoxin Recovery Likelihood Tool

In accordance with the LER Risk Based Approach for investigational medicinal products described previously, the assessment using the Theoretical Low Endotoxin Recovery Likelihood Tool was performed for the vaccine DP in two steps:

1 - Evaluation of the formulation components using the theoretical LER likelihood assessment on the vaccine DP.

2 – Final theoretical likelihood rating is assigned based on the theoretical LER likelihood tool.

Five potentially LER causing components were identified in the vaccine DP formulation:

- Surfactant
- Strong divalent metal ion chelator: EDTA
- Phosphate mono- and bivalent
- Weaker divalent metal ion chelator
- Serum/protein(s)

The final LER likelihood rating based on the evaluation using the Sanofi-developed likelihood tool for the vaccine DP was categorized as 'Very High' due to the presence of Surfactant and EDTA. FDA presented drug sponsor-generated BLA data indicates this combination of components aligns with an approximate 30% chance of LER when evaluated using CSE or RSE spike-recovery studies.

Study Design

The LER study was conducted using the "Multi-aliquot" mode: one large sample volume and an identical LRW (Low Reagent Water) control were spiked with 5 IU/mL of RSE to target the mid-point of the standard curve. They were then aliquoted at the start of the study (Day 0) on the same day prior to storage at +5°C±3°C in the vaccine product final containers. Afterwards, analyses were performed on these aliquots at the selected time points as per our internal kinetic quantitative chromogenic LAL SOP and as per the conditions defined during the vaccine DP routine validation (diluent, standard range, working dilution, Lonza reagents).
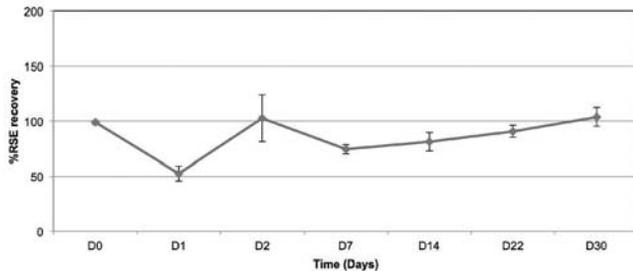
Product was tested at Day 0, 1, 2, 7, 14, 22 and 30 after storage at +5°C±3°C. Lot was tested as 3 replicates in duplicate (ie 6 times) by two analysts.

The table below summarizes the results of the study.

	Ave% Recovery spiked product – replicate 1	Ave% Recovery spiked product – replicate 2	Ave% Recovery spiked product – replicate 3
Table 7. Summary of results of the LER study performed on the vaccine DP			
Day 0	100	97	100
Day 1	47	60	50
Day 2	93	88	127
Day 7	73	71	79
Day 14	73	81	90
Day 22	86	97	90
Day 30	113	103	96

Analysed data are presented in the following figure.

The percentage of average recovery of RSE did not drop less than 50% for two or more consecutive time points: the 3 replicates of the vaccine DP did not exhibit LER.

Assayable endotoxin content over time in undiluted product DP was demonstrated stable over 30 days at +5°C±3°C after sampling.

Discussion of Case Study Results

Per the likelihood tool, a very high likelihood (~30% chance) of LER was established for this vaccine DP. However, we demonstrated experimentally that this formulation does not exhibit LER. To better understand this phenomenon; the vaccine formulation can be further analyzed.

The phosphate content was comparably high in this vaccine formulation and there was a relatively low concentration of strong divalent metal ion chelator.

On one hand, the presence of arginine may have blocked some phosphates and EDTA due to its positively charged amine groups. On the other hand, serum/protein(s) may have a stabilizing effect by absorbing surfactants or other amphiphilic compounds such as the type of surfactant used in this formulation. Thus, they could not form mixed, low-activity aggregates with LPS and endotoxin. Generally, certain proteins can promote LER by absorbing LPS or bivalent ions; others can inhibit LER by absorbing surfactants or chelating agents. It depends on the individual protein structure (charged and hydrophobic moieties on surface). Especially, the high concentration of proteins contained in our vaccine DP could be stabilizing against LER.

Taken together, the physico-chemical properties of the proteins were the suspects for the observed lack of LER.

It is important to remember that the estimation of LER likelihood described here is only one component of an overall holistic strategy designed to satisfy regulatory requirements. It does not replace the necessity of a formal LER study for new products, regardless of the result of the evaluation, as demonstrated here.

Conclusion

LER is currently a hot topic in the industry, but even highly theoretically likely LER formulations such as EDTA with a surfactant do not always result in experimentally confirmed LER. Properly designed and correctly interpreted experimental studies remain critical in determining if LER is present in a given formulation. Existing commercial products, with demonstrated low endotoxin ingress risk, documented effective controls, and strong patient safety records, can be appropriately managed using a holistic risk-based approach.

Holistic strategies, toolkits, and emerging guidance is finally advancing the LER topic past the theoretical debates and toward a common ground more useful for practical application.

Acknowledgements

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