COMMENTARY

Endotoxin Limits in Formulations for Preclinical Research

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ABSTRACT: This brief commentary discusses a review of the current status on endotoxin limits, a critical parameter, for formulations to be administered to animals. The endotoxin units set by United States Pharmacopoeia (USP), and the techniques specified by USP for endotoxin testing are described. Endotoxin limits for preclinical research animal models were derived based on the threshold pyrogenic human dose of 5 E.U. per kg. The limits calculated would act as a guideline for endotoxin limits in preclinical species. A quick reference chart for endotoxin limits is included to provide a guideline for endotoxin limits for animal models used in preclinical research. Derivation of endotoxin limits from K/M for doses and animal models not included in the chart could be calculated as described.
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INTRODUCTION

Researchers in preclinical research are often perplexed due to the variation in acceptable endotoxin limits set by different groups for this critical parameter. Although the United States Pharmacopeia (USP) and United States Food and Drug Administration (USFDA) have set guidelines for scores of drugs, raw materials, and articles, there is little mention of endotoxin limits for preclinical animal models commonly used in research. Further complicating the endotoxin standards is a lack of harmonization in the expression of endotoxin limits. A nationwide survey of the biotechnology industry was conducted by the Quality Assurance/Quality Control task group of the Parenteral Drug Association regarding endotoxin and pyrogen testing and control.[1] The most obvious lack of standardization that emerged from this survey was absence of a standard limits format in release testing. The units used for release testing varied not only from company to company, but also among different sample types within the same manufacturing process. This made comparisons difficult in regard to both different companies and process steps within a single company.[1] This brief review will discuss the endotoxin units set by USP, the techniques specified by USP for endotoxin testing, calculation of the endotoxin limits for animal models commonly used in preclinical research and a ready reference chart for endotoxin limits in small animal models.

ENDOTOXIN LIMITS—UNITS, TESTING, AND DERIVATION

The test for measuring endotoxin limits currently recognized by USP 30[2] is the Limulus Amebocyte

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Lysate (LAL) Test. The LAL test is otherwise known as the Bacterial Endotoxins Test. Endotoxin limits are expressed as USP EU (Endotoxin units).

The limits are also expressed as nanograms and International Units (IU). USP 30 gives a conversion of USP EU to IU, which is, "One USP-EU is equal to one IU of endotoxin". The conversion from endotoxin units to nanograms varies depending on the source of the endotoxin. USFDA's sub lot of the International standard, EC-6, has been assigned a potency of 10 EU/ng.[3] Expressing endotoxin concentrations in EUs avoids the issues of different potencies of different endotoxins and also focuses attention on the activity of the endotoxin.[4] EC-6 consists of endotoxin extracted from the cells of *Escherichia coli* 0113.[4] This endotoxin standard, referred to as the harmonized standard, has been adopted as a common standard by the US, World Health Organization and the European Pharmacopoeia.[4]

The endotoxin assays specified by the USP 30 are based on two primary techniques, gel clot technique and photometric technique. The turbidimetric method and the chromogenic method are photometric techniques. In case of discrepancies between techniques, USP bases its decision on the gel clot technique, unless otherwise specified. In the gel-clot techniques, the reaction endpoint is determined from dilutions of the material under test in direct comparison with parallel dilutions of a reference endotoxin, and quantities of endotoxin are expressed in USP Endotoxin Units (USP-EU) [note—One USP-EU is equal to one IU of endotoxin].[2] The gel clot technique has a twofold error built into the test and the USP and USFDA guidelines allows a user to be within a twofold error margin and recommends taking the higher endotoxin value when there is variability between gel clot assays.[5] To define endotoxin limit, the USP 30[2] states that, "The endotoxin limit for parenteral drugs, defined on the basis of dose, is equal to K/M, where K is the threshold human pyrogenic dose of endotoxin per kg of body weight, and M is equal to the maximum recommended human dose of product per kg of body weight in a single hour period." *The USP 30 further states that*, *"K* is 5 USP-EU/kg for any route of administration other than intrathecal (for which K is 0.2 USP-EU/kg body weight). For radiopharmaceutical products not administered intrathecally the endotoxin limit is calculated as $175/V$, where V is the maximum recommended dose in mL. For intrathecally-administered radiopharmaceuticals, the endotoxin limit is obtained by the formula $14/V$. For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is K/M, where $K = 5$ EU/kg and M is the (maximum dose/m^2/h $\times$ 1.80 m^2)/70 kg."

The K/M formula established for humans by US FDA and stated by the USP 30 has been used here and extrapolated to calculate the endotoxin limits for various animal models.[6] The threshold pyrogenic human dose of 5 EU/kg is assumed to be threshold pyrogenic dose for individual preclinical species. The endotoxin units have been calculated as EU per mg and EU per mL based on the state of the material assayed. Mouse is a common animal model used in preclinical research and the endotoxin limit for a mouse model was calculated as follows (Tab. 1).

To calculate M, we need the maximum dose of product per hour and the body weight of the mouse model. The body weight of the mouse was taken as 30 g, which would be 0.03 kg. Taking 10 μg (0.01 mg)/h as an example of a mouse dose, and extrapolating M (equal to the maximum recommended human dose of product per kg of body weight in a single hour period) to a mouse, M would be 0.333 (0.01 mg/0.03 kg). Substituting 5 EU/kg for K and 0.333 mg/kg/h, K/M would be 15 EU/mg. Thus if an investigator plans to dose a mouse animal model at 10 μg/h, the drug should have an endotoxin of less than 15 EU per mg for administrations other than intrathecal route. In the same example, if 10 μg is the maximum daily mouse dose, then M would be 0.013875 (0.01 mg/0.03 kg/24 h) and the K/M would be 360 EU/mg. The endotoxin limit has increased from 15 to 360 EU/mg based on the dosage per hour.

It is also important, however, to calculate the endotoxin limit per volumetric dose (Tab. 1). The volumetric dose would comprise of one or more drugs and excipients and would contribute

Table 1. Calculating Endotoxin Limit for a Mouse for a Drug Dose in mg and mL

	Drug (mg)	Drug (mL)
Weight of mouse	0.03 kg	0.03 kg
Dose in mg/h	0.01 mg/h	0.100 mL/h
M (mg/kg/h)	0.3333 mg/kg/h	3.3333 mL/kg/h
K	5 EU/kg	5 EU/kg
K/M	15 EU/mg	1.5 EU/mL

Table 2. Endotoxin Limits for Drugs in mg/kg for Commonly Used Animal Models in Preclinical Research at Typical Doses

Model	Body Weight (kg)	Dose (mg/h)	EU/mg
Mouse	0.03	0.001	150
		0.010	15
		0.025	6
Gerbil	0.09	0.001	450
		0.010	45
		0.025	18
Rat	0.45	0.001	2250
		0.010	225
		0.025	90
Rabbit	4	0.010	2000
		0.025	800
		0.050	400
Monkey	8	0.250	160
		0.500	80
		1.000	40
Baboon	12	0.250	240
		0.500	120
		1.000	60

to the total endotoxin content of the formulation. For a 100 μL (0.100 mL) daily dose, *M* would be 0.139 (0.100 mL/0.03 kg/24 h) and *K*/*M* would be 36 EU. The endotoxin limit for a 100 μL daily dose would be 36 EU/mL and that of a 100 μL/h dose would be 1.5 EU/mL.

Table 3. Endotoxin Limits for Drugs in mL/kg for Commonly Used Animal Models in Preclinical Research at Typical Doses

Model	Body Weight (kg)	Dose (mL/h)	EU/mL
Mouse	0.03	0.050	3.00
		0.100	1.50
		0.200	0.75
Gerbil	0.09	0.050	9.00
		0.100	4.50
		0.200	2.25
Rat	0.45	0.050	45.00
		0.100	22.50
		0.200	11.25
Rabbit	4	0.10	200
		0.20	100
		0.50	40
Monkey	8	0.10	400
		0.20	200
		0.50	80
Baboon	12	0.10	600
		0.20	300
		0.50	120

The endotoxin limits are presented as a quick reference chart (Tabs. 2 and 3) to provide a guideline for preclinical research workers. Table 2 lists the endotoxin limits for a drug by weight for animal models most used in preclinical research. Three typical doses used for each animal model has been taken for calculating the endotoxin limits and the calculation is based on *K*/*M* formula as shown in Table 1 for a mouse model. The endotoxin limits for a drug by volume are included in Table 3 and three typical doses used in these animal models have been taken as in Table 2.

Table 1 can be used as a guide to calculate "*M*" for a dose not included in Tables 2 and 3 and the endotoxin limits (*K*/*M*) calculated by using the calculated "*M*" as an input and substituting 5 EU/kg for "*K*". Care has to be taken to ensure "*M*" has the units of mg/kg/h or mL/kg/h before substituting in *K*/*M* to calculate the endotoxin limits.

CONCLUSION

To conclude, having a clear perspective of this critical parameter would enable a scientist to design formulations with acceptable endotoxin limits. LAL Test is the currently recognized test by USP 30 for measuring endotoxin limits. Endotoxin limits are expressed as USP EU (Endotoxin units). The gel clot technique specified by USP is the recommended technique for the determination of endotoxin limits. Due to the twofold error built into the test, the USP and USFDA recommend taking the higher endotoxin value when there is variability between gel clot assays. The threshold pyrogenic human dose of 5 EU/kg is assumed to be threshold pyrogenic dose for individual preclinical species. The limits calculated would act as a guideline for endotoxin limits in preclinical species. A quick reference chart (Tabs. 2 and 3) for endotoxin limits is included to provide a guideline for endotoxin limits for animal models used in preclinical research. Derivation of endotoxin limits from *K*/*M* for doses and animal models not included in the chart could be calculated as described above in Table 1.

REFERENCES

1. The quality assurance/quality control task group. 1990. Parenteral Drug Association, Current practices in endotoxin and pyrogen testing in biotechnology. J Parenter Sci Technol 44:39–45.

2. Bacterial Endotoxins Test. USP 30, http://www.uspnf.com/uspnf/pub/index?usp=30&nf=25&s=0.
3. Cooper JK. The Necessity of Reliable Endotoxin Standards http://www.criver.com/flex_content_area/documents/qc_lr_n_reliable_end_standards.pdf#search=%22charles%20river%20and%20RSE%22.
4. Dawson ME. 1997. Harmonization of endotoxin standards and units. LAL Update 15:2–3.
5. Variability in the Endotoxins test or Limulus Amebocyte Lysate (LAL)Test. Endosafe times, Vol. 12 No. 1, August 2006 http://www.criver.com/flex_content_area/documents/qc_lr_n_et_LAL_test_variability_Aug_06.pdf.
6. Weary ME. 1990. Understanding and setting endotoxin limits. J Parenter Sci Technol 44:16–18.