REPEATED DOSE IN VIVO ORAL TOXICITY STUDY TO TEST LONG-TERM EFFECTS OF THE MYCOTOXIN ENNIATIN B IN MALE AND FEMALE CD-1 MICE: FOCUS ON HISTOPATHOLOGICAL DATA FOR THE NOAEL DEFINITION

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BACKGROUND

Enniatin B (ENN-B) is an emerging mycotoxin, secondary product of Fusarium fungi, detected in food and feed, mainly in cereal grains. ENN-B showed antibacterial, antihelmintic, antifungal, herbicidal, and insecticidal activities, the main mechanism of action being its ionophoric characteristics. ENN-B showed a potent cytotoxic activity in several mammalian cell lines; despite this, European Food Safety Authority (EFSA) stated that acute exposure does not indicate concern for human health. Nevertheless, insufficient data exist to establish a tolerable daily intake and/or an acute reference dose given the lack of relevant toxicity data following chronic exposure and a risk assessment was not possible.

No-Observed-Adverse-Effect-Level (NOAEL)
vs
Benchmark dose (BMD) approach

Advantages and limitations of the NOAEL and BMD methods.

BMD advantages	NOAEL limitations
• Not limited to experimental doses	• Highly dependent on dose selection
• Less dependent on dose spacing	• Highly dependent on sample size
• Appropriately accounts for variability and uncertainty resulting from study quality	• Does not account for variability and uncertainty in the experimental results (e.g., does not account for study quality appropriately)
• Takes into account the shape of the dose–response curve and other related information	• Dose–response information (e.g., shape of dose–response curve) not taken into account
• Corresponds to consistent response level and can be used to compare results across chemicals and studies	• Does not correspond to consistent response levels for comparisons across studies
• Flexibility in determining biologically significant rates	• A LOAEL cannot be used to derive a NOAEL
BMD limitations	**NOAEL advantages**
• Ability to estimate BMD may be limited by the format of data presented	• Can be used when data is not amenable for BMD modeling
• Time consuming	• Easy to derive
• More complicated decision making process	• Has been the standard method for deriving a POD for decades (e.g., is familiar to most risk assessors)

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link https://shiny-efsa.openanalytics.eu/app
Maranghi F. et al (2018) In vivo toxicity and genotoxicity of beauvericin and enniatins. Combined approach to study in vivo toxicity and genotoxicity of mycotoxins beauvericin (BEA) and enniatin B (ENNB). EFSA Supporting publication 2018:EN-1406
Davis JA et al (2010) Introduction to benchmark dose methods and U.S. EPA's benchmark dose software (BMDS) version 2.1.1. Toxicol Appl Pharmacol. 2011 Jul 15;254(2):181-91.

EXPERIMENTAL DESIGN

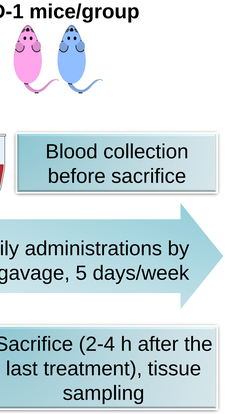
The study was conducted according to the European Community Council Directive 2010/63/UE the Italian Law 4 March 2014 n. 262 and the OECD Principles on GLP.

Dose level calculation

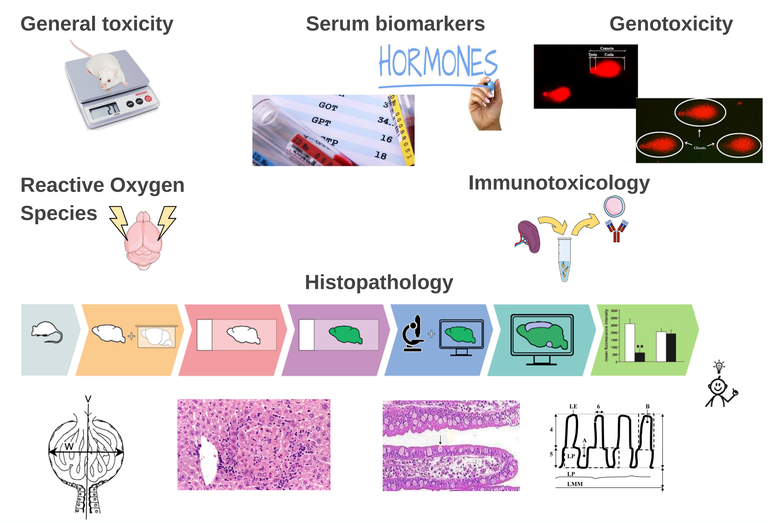
Starting point
LD50 ➡ 350 mg/kg b.w.

CTRL ➡ olive oil+6%DMSO
ENN-B ➡ 0.18, 1.8, 18 mg/kg b.w. per day

0.18 mg/kg bw per day is comparable with the rough estimate for a LOAEL for short-term exposure - 0.09 to 0.17 mg/kg b.w. - for the therapeutic treatment with Locabiotal®



Endpoints



(key) RESULTS for NOAEL

ORGAN/Effect		Males				Females			
		Olive oil+DMSO 6%	ENNB0.18	ENNB1.8	ENNB18	Olive oil+DMSO 6%	ENNB0.18	ENNB1.8	ENNB18
DUODENUM	*N of mice examined*	6	7	7	6	7	7	6	7
Enterocytes vacuolation	0	6	7	7	1	5	3	2	3
	1				2		2	1	
	2				3	2	2	2	4
	3							1	
Total Finding Incidence		0	0	0	5#	2	4	4	4

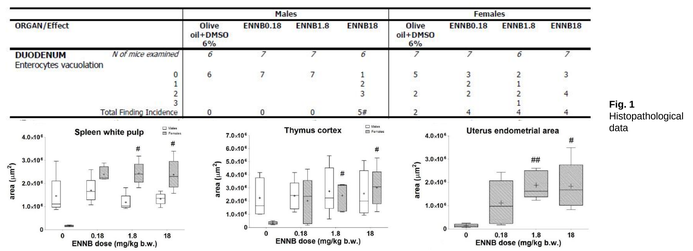


Fig. 1 Histopathological data

Sex	NOAEL (mg/kg b.w./day)	LOAEL (mg/kg b.w./day)	Effect
Male mice	1.8	18	Enterocyte vacuolization in duodenum Increased ROS and GSH brain levels
Female mice	0.18	1.8	Histopathological effects on thymus, uterus, spleen

BMD (on the same endpoints)

Endpoint	Male mice		Female mice	
	BMDL (mg/kg b.w./day)	**BMDU** (mg/kg b.w./day)	**BMDL** (mg/kg b.w./day)	**BMDU** (mg/kg b.w./day)
Vacuolization in duodenum	15.1	17.8		
Thymus cortex area			1.00E-06	0.065
Uterus endometrial area			1.35E-06	0.075
Spleen white pulp area			2.39E-06	14

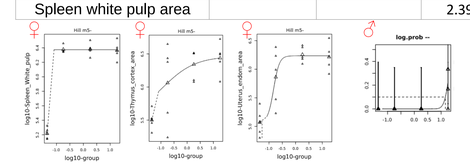


Fig. 2 - BMD fit curves for spleen white pulp area, thymus cortex area and uterus endometrial area in females and vacuolization in duodenum in males.

CONCLUSIONS

ENN-B treatment affected spleen, brain and thyroid in both sexes, whereas thymus, kidneys, adrenals and reproductive system were affected in female mice only, and duodenum in male mice only.

The data generated by the present study could be used to propose a NOAEL/LOAEL according to the traditional toxicological approach, and BMDL values according to the BMD approach. Indeed, the BMD approach is a scientifically more robust method and it is of particular value taking into account directly the shape of the dose-response curve.

NOAEL vs BMD

In male mice, BMDL is 10-times higher than NOAEL, resulting comparable to LOAEL. In female mice, the ratio between BMDU and BMDL is too high to suggest a reliable result.