



CODA –CERVA

**Belgian National Reference Laboratory
for Mycotoxins in Food and Feed**

**Report on the 2010 Proficiency Test for the
Determination of Zearalenone in wheat draff**

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Summary

A Proficiency Test (PT) on the determination of zearalenone (ZEA) in wheat draff was conducted with 9 laboratories in Belgium, one of which provided two separate sets of results obtained with two different analytical methods. The sample was a certified reference material (naturally contaminated wheat draff, ref 31/106) purchased from Bipea.

The assigned value ($X_{\text{ref}} = 98 \mu\text{g/kg}$) and the standard uncertainty on this value ($u_{\text{ref}} = 11 \mu\text{g/kg}$) were provided by Bipea. The standard deviation for proficiency assessment ($\sigma_p = 24.5 \mu\text{g/kg} = 25\%$) was taken as half the tolerance chosen by Bipea, and is slightly higher than the standard deviation calculated using the modified Horwitz function (22%).

The z-scores were satisfactory for 8 results ($-2 < z < 2$) and questionably low ($-3 < z < -2$) for the other two results.

The zeta-scores were satisfactory for 7 results, but too low ($-5.8 < \text{zeta} < -2.8$) for the three lowest results. The main reason for the underestimations of uncertainty of measurement seems to be that (negative) biases had been underestimated (overestimation of IAC recovery).

Introduction

Zearalenone is a white crystalline compound, insoluble in water but soluble in various organic solvents. This toxin is stable during storage as well as milling, processing and cooking of food. It does not degrade at high temperatures.

Due to its structure, ZEA binds to mammalian oestrogen receptors. So ZEA induces oestrogenic effects on mammals and interferes with conception, ovulation, implantation, foetal development and viability. Beside its oestrogenic activity, ZEA also possesses anabolic activity.

ZEA was also regarded as a causative agent in epidemics of premature thelarche in girls (Puerto-Rico, 1978 and 1981, South-eastern Hungary 1997) but there is a lack of adequate information.

Occurrence : Zearalenone is a mycotoxin produced by several field fungi of the genus *Fusarium* such as *F.graminearum*, *culmorum*, and *cerealis*. Those fungi infect cereals pre-harvest in the fields during blooming. Growing and production of toxins may also occur post-harvest under poor storage conditions (moisture>22%).

Because the spores of *Fusarium* are ubiquitous, cereals as barley, oats, wheat, rice, maize are susceptible to contamination with zearalenone. It occurs in both temperate and warmer climate zones.

According to the ZEA levels found in feed, transmission of ZEA into tissues and milk of ruminants does not pose a significant health risk. Even with cows fed at a 165 mg/day intake, no detectable residues could be found in the milk. Because of the very high LD50, ZEA is more a fungal non steroidal hormone (oestrogen) than a direct mycotoxin.

ZEA is regulated within the European union:

Maximum levels in foodstuffs: wheat, durum wheat, oats, barley, maize, baby food...

-(<http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF>)
COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[Official Journal of the European Union, L 364/5-24, 20.12.2006](http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

-(<http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:255:0014:0017:EN:PDF>)
COMMISSION REGULATION (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products

[Official Journal of the European Union, L 255/14-17, 29.9.2007](http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:255:0014:0017:EN:PDF)

Maximum levels in animal feed

-(<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF>)
COMMISSION RECOMMENDATION (2006/576/EC) of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

[Official Journal of the European Union, L 229/7-9, 23.8.2006](http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF)

Tolerable daily intake

-(http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_contam06_ej89_zearalenone_v3_en1,2.pdf?ssbinary=true)

-Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Zearalenone as undesirable substance in animal feed, The EFSA journal 89, 1-35 (2004)

Test material and instructions to participants

There were nine participants, including the NRL. They were all laboratories approved by the Belgian Federal Agency for the Safety of the Food Chain (FASFC).

The test material was a certified reference material (naturally contaminated wheat draff, ref 31/101, 2009) purchased from Bipea. The original bag, containing approximately 700 g of wheat draff test material, was opened, remixed and distributed in bags of approximately 55g. Those were labelled and kept frozen until they were dispatched to the participants on June 25, 2010. Each participant received one bag.

The participants were invited to follow their routine procedures for the determination of zearalenone and report (see annexes) :

- the values of two independent measurements, corrected for recovery
- the recovery factor used for correction
- an estimate of their uncertainty of measurement ($u_{k=1}$ or $U_{k=2}$) and the coverage factor (k) used
- a short description of the purification and measurement method

The results were to be reported on a form provided in attachment, in the same manner (eg. number of significant figures) as when reporting to customers.

Reference values

The reference value X_{ref} , and its standard uncertainty u_{ref} were respectively 98 $\mu\text{g}/\text{kg}$ and 11 $\mu\text{g}/\text{kg}$, as reported by Bipea. They are the robust mean and the robust standard deviation of the results of 30 out of the 38 laboratories having reported a result for zearalenone in their PT nr 31/106. The standard deviation for proficiency assessment ($\sigma_p = 24.5 \mu\text{g}/\text{kg} = 25\%$) was taken as half the tolerance chosen by Bipea for their PT (49 $\mu\text{g}/\text{kg}$). This is slightly higher than the standard deviations calculated from the Horwitz equation (22.7%) and the Horwitz-Thompson equation (22%). (M. Thompson (2000) Analyst, 125, 385-386).

Results and discussion

The participants reported their results before deadline (Aug. 31). One of them did not give an answer.

All participants reported two measurement values, and all except two reported their recovery. All provided an estimate of their measurement uncertainty, and all reported that the coverage factor for this estimate was $k=2$.

Four laboratories used immuno-affinity clean-up (IAC). Three of these used fluorimetric detection (LC-FL), one used MS-MS detection (UPLC-MS). Of the remaining five laboratories, four used LC-MS-MS without IAC clean-up, and one applied a C18 clean-up before LC-MS-MS.

Individual laboratory performance is expressed in terms of z and zeta scores in accordance with ISO 13528 and the International Harmonised Protocol.

$$Z = \frac{X_{lab} - X_{ref}}{\sigma_p}$$

$$Zeta = \frac{X_{lab} - X_{ref}}{\sqrt{u_{ref}^2 + u_{lab}^2}}$$

where

X_{lab} is the measurement result reported by a participant

X_{ref} is the assigned value

σ_p is the "standard deviation for proficiency assessment"

u_{ref} is the standard uncertainty on the reference value

u_{lab} is the standard uncertainty reported by a participant

(all in $\mu\text{g}/\text{kg}$ – note that for the calculation of the zeta-scores the values of u_{lab} in $\mu\text{g}/\text{kg}$ were recalculated from the u_{lab} as reported in %, in order to avoid errors due to the rounding of the u_{lab} as reported in $\mu\text{g}/\text{kg}$)

The laboratory codes were attributed by increasing value of z-score. Each lab code was communicated confidentially to the corresponding participant. The results reported by the participants and the z- and zeta-scores are summarized in table 1 and figure 1.

Visual examination of figure 1a ("kernel plots") shows that the distribution of results is distinctly skewed, with four low results and six within the uncertainty range of the reference value. Strikingly, the four low results were those obtained after IAC clean-up.

Most of the results were in the acceptable range ($-2 < z < 2$). Only the two lowest were in the questionable range ($-3 < z < -2$).

Most laboratories (L04 to L10) also obtained good zeta-scores, i.e. their estimates of their measurement uncertainty is such that the 95% confidence interval around their result includes the reference value, or at least part of the 95% confidence interval around it (see figure 1b).

The other laboratories, L01 to L03, underestimated their measurement uncertainty and obtained zeta-scores between -5.8 and -2.8. In these three cases the reported MU seem realistic at first sight (25% to 34%, $k=2$) but are smaller than the deviations from the reference value (-38% to -71%). As already recalled in the 2009 OTA PT report, for the end-user, zeta-scores lower than -2, such as those obtained by laboratories L01 to L03, mean a risk of wrong decisions due to "false negatives" or "false compliants", i.e. of accepting a lot of food or feed which should have been rejected.

Table 1 : Values as reported by the participants and scorings calculated by the organiser

lab code	data as reported by the participants :						calculations :			
	result 1 (µg/kg)	result 2 (µg/kg)	recovery (%)	uncertainty (µg/kg)	uncertainty (%)	k (coverage factor)	average (µg/kg)	deviation from Vref	z-scores	zeta-scores
L01	29.7	26.8	102.8%	9.6	34%	2	28.3	-71%	-2.85	-5.81
L02	49	48		(16)	32%	2	48.5	-51%	-2.02	-3.68
L03	64.4	56.1	100% - 104%	15.1	25%	2	60.3	-39%	-1.54	-2.83
L04	70.6	61.7	89.8%	22	35%	2	66.2	-33%	-1.30	-1.99
L05	76		107%	18	24%	2	76.0	-22%	-0.90	-1.54
L06	73.5	97.6	91.5%	18.9	22%	2	85.6	-13%	-0.51	-0.86
L07	88.1	92.3	97%	(23)	25%	2	90.2	-8%	-0.32	-0.50
L08	91	98	101% - 95%	27	29.04%	2	94.5	-4%	-0.14	-0.20
L09	96	100		21	21%	2	98.0	0%	0.00	0.00
L10	91	112	101%	(47)	46%	2	101.5	4%	0.14	0.14

Notes :

- when not reported by the participants, the uncertainties in µg/kg were calculated from the average results and the uncertainties in % ; these are given between parentheses
- two participants reported different recoveries for the two results. Laboratory LO9 stated that no correction for clean-up was needed because they used an internal ¹³C standard.

Figure 1a :
Zearalenone (ZEA)
in wheat draff ($\mu\text{g}/\text{kg}$),
kernel density plot

(vertical lines :
solid : X_{ref}
dashed : $X_{\text{ref}} \pm 2 \cdot u_{\text{ref}}$
dotted : $X_{\text{ref}} \pm 2 \cdot \sigma_p$

with
 $X_{\text{ref}} = 98 \mu\text{g}/\text{kg}$;
 $u_{\text{ref}} = 11 \mu\text{g}/\text{kg}$
 $\sigma_p = 24.5 \mu\text{g}/\text{kg}$

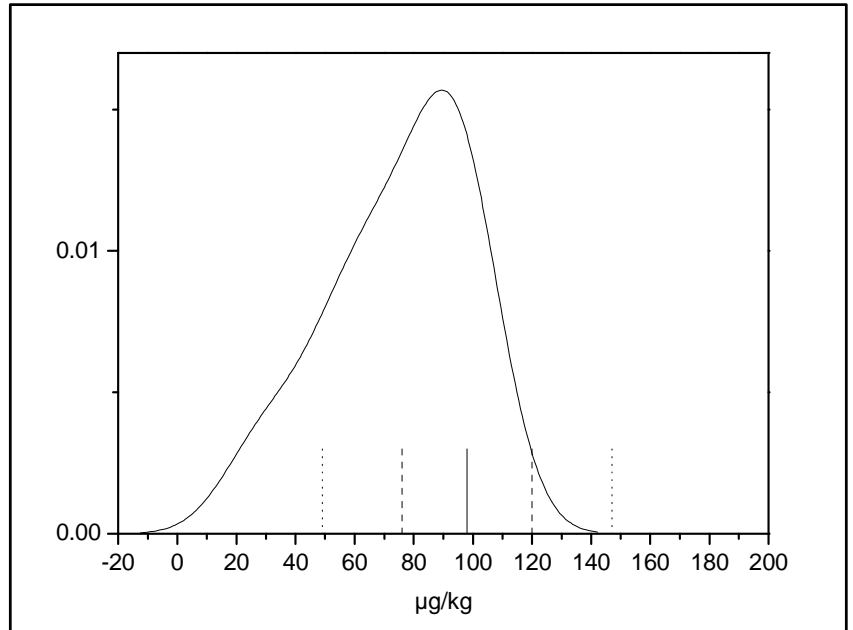


Figure 1b :
Results and
95% confidence intervals
(expanded uncertainty),
as reported by participants

(horizontal lines :
solid : X_{ref}
dashed : $X_{\text{ref}} \pm 2 \cdot u_{\text{ref}}$
dotted : $X_{\text{ref}} \pm 2 \cdot \sigma_p$)

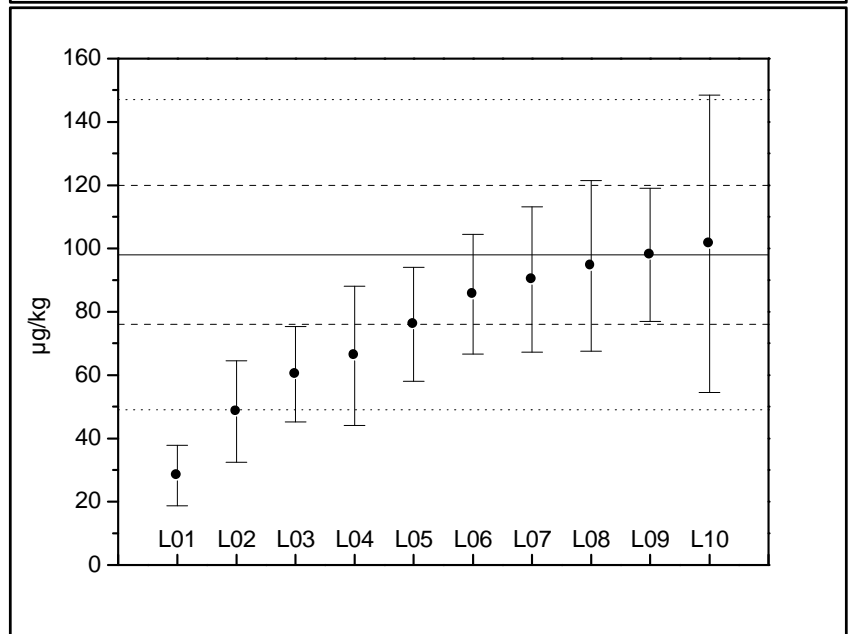
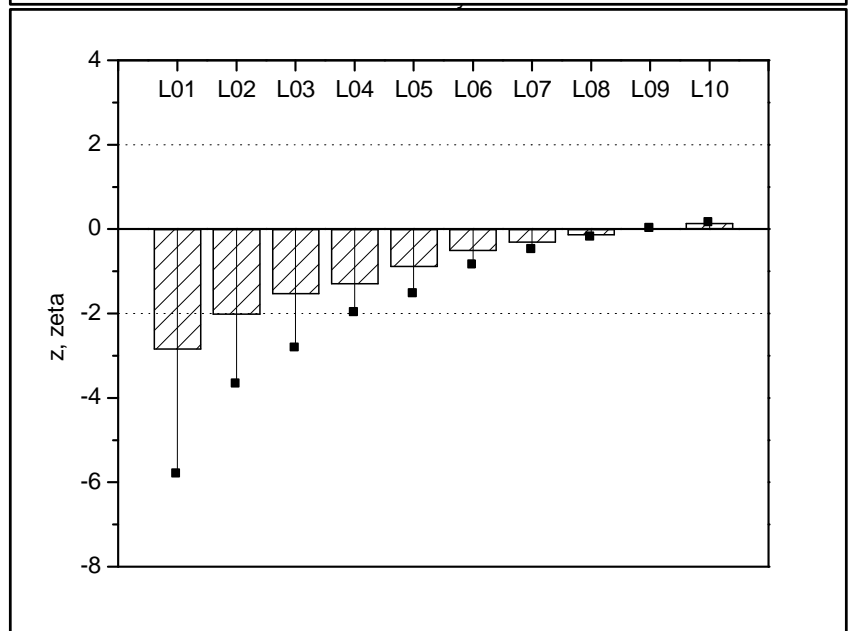


Figure 1c : z- and zeta-scores

$$z = (X_{\text{lab}} - X_{\text{ref}}) / \sigma_p$$

$$\text{zeta} = (X_{\text{lab}} - X_{\text{ref}}) / \sqrt{u_{\text{ref}}^2 + u_{\text{lab}}^2}$$



Annexes

List and addresses of the participating laboratories (by alphabetical order) :

Organisation	Address	
CER Marloie	Rue du Point du Jour, 8	B-6900 Marloie
CERVA-CODA	Leuvensesteenweg, 17	B-3080 Tervuren
ECCA NV	Ambachtsweg, 3	B-9820 Merelbeke
FLVVT	Leuvensesteenweg, 17	B-3080 Tervuren
FLVVG	Braemkasteelstraat, 59	9050 Gentbrugge
Fytolab	Technologiepark, 2/3	B-9052 Zwijnaarde
LABUG, Lab voor Bromatologie	Harelbekestraat, 72	9000 Gent
OLEOTEST	Lage weg, 427	B-2660 Antwerpen
SGS-Belgium	Polderdijkweg, 16 Haven 407	B-2030 Antwerpen

Letter to the participants :



V A R
VETERINARY AND AGROCHEMICAL RESEARCH CENTRE



your reference

our reference

your contact: Jean-Claude Motte
tél.: 02/769 22 80
jemot@var.fgov.be

date
june, 26th 2010

SUBJECT: Ring Test ZEA in Wheat

ANNEXES:

Dear Participant,

In the annexes you will find:

- annexe 1 : description of the sample and instructions about the results to be sent back.
- annexe 2 : the test-sample (grinded feed grains)
- annexe 3 : Receipt form of the sample
- annexe 4 : results form

Annexe 3 is a receipt form. Please fill the form and return it us by FAX (FAX n°: 02/769.23.05) as soon as you receive the sample.

Annexe 4 is for the results form of the analysis. Please enter here the name of the analyst, the name of your laboratory, the sample code, for each replicate the calculated concentration for ZEA corrected for recovery and the recovery. **Do not forget to fill in the uncertainty and the coverage factor.**

Also give a short description of the used method as for test-report for both purification step and measurement (LC...).

After checking all the data you have entered, **first submit** the form electronically to the proposed Email. **Afterwards** you may **save** the form on your PC, **print** it, **sign** the hardcopy and **fax** or **mail** it to the address shown on the form.

The **deadline** for **reporting** back results is **August 31th, 2010** (we give a longer period of ten weeks taking into account the summer holidays).

As I am the project leader for this inter-laboratory comparison please do not hesitate to contact me in case of questions or doubts.

Your participation is free of charge.

Yours sincerely,

Dr Sc. Jean-Claude Motte

Remember: So far this test is organised in collaboration with the FASFC (AFSCA-FAVV), please note that you have the obligation to participate.

----- Scientific research at the service of safe food production and animal health -----

Central office
Veterinary departements:
Groeselenberg 99, B-1180 Brussels
Tel. +32 (0)2 379 04 00
Fax +32 (0)2 379 06 70 (general)
+32 (0)2 379 04 01 (director)
+32 (0)2 379 06 64 (dispatching)

Agrochemical departements:
Leuvensesteenweg 17
B-3080 Tervuren
Tel. +32 (0)2 769 22 00
Fax +32 (0)2 769 23 05

Experimental centre
Kerklaan 68
B-1830 Machelen
Tel. +32 (0)2 251 33
26
Fax +32 (0)2 251 00
12



be

e-mail info@var.fgov.be - http://www.var.fgov.be



Ring-Test “Zearalenone”

Annexe 1: description

1. Introduction

The participation in proficiency testing schemes is an essential element of the quality-management-system of every laboratory testing food and feed. The implementation of proficiency tests enables the participating laboratories to prove their own analytical competence under realistic conditions. At the same time they receive valuable data regarding the validity of the particular testing method.

The purpose of VAR is to offer proficiency tests for selected parameters in concentrations with practical relevance. Realisation and evaluation of the present proficiency test is based on the principles of the ISO guide 43 (1997).

2. Realisation

2.1. Test material

The test material is grinded feed grains obtained in bulk from an accredited society known for their ring test organisation. The sample is sent in about 52gr portion stocked at -18°C. Mailing and manipulation may occur at room temperature.

2.2. Test

One portion of test material will be sent, from CERVA-Tervuren, to every participating laboratory in the last week of June 2010. The testing method should be the laboratory routinely method used for ZEA determination. The results should be sent on 31th of august the latest.

2.3. Results

The participants are requested to submit 2 independent measurements. The results will be submitted on the standard form (annexe 4). The returned results will be: 1-results corrected by recovery for both replicates; 2-recovery; 3-average and 4-uncertainty (u) with the coverage factor. The results should be reported in the same way (e.g.: number of significant figures) as usually reported for the customers.

Please also report the results with technique, short method description and uncertainty (u) information in the allocated space on the results form.

Please do not report uncertainty on each individual measurement.

Keep care: do not forget to state your coverage factor.

Receipt form :



RING – TEST ZEARALENONE VAR 06/2010

Please send to : J.C.Motte , FAX n° 02/769.23.05

Annexe 3: receipt form

Name of the Laboratory :	
Name of the analyst :	

Sample number :	_____ - _____		
Date of reception of the sample :			
State of the received sample :	Good	Bad	Open

Date	stamp	Signature

Results form :



RING – TEST ZEARALENONE VAR 06/2010
 SUBMIT RESULTS TO : jemot@var.fgov.be

Annexe 4: results form

DEADLINE :	31 th of august 2010
Name of the Laboratory :	
Name of the analyst :	

Parameter	Results corrected for recovery	Units	Recovery as %	uncertainty (u)		
				Units		
VAR- testnumber	-					
<i>Zearalene</i>				μg/kg	%	k
Replicate 1		μg/kg				Coverage factor
Replicate 2		μg/kg				

	Yes	No
Recovery in the same matrix		

Short method description as for test-report :	
Purification :	
Measurement:	

Datum :

Stamp :

Signature :