

## Joint Research Centre (JRC)

# Analytical Aspects of Mycotoxin Binders

Anna Kolossova & Joerg Stroka



<http://www.jrc.ec.europa.eu/>

**Legal background**

**Type of Binders, what are they?**

**Proposed mode-of-action in animals**

**Binders available to test in the study**

**Design/outline of study and evaluation**

**Do they have an effect on analytical methods?**

**REGULATION (EC) No 1831/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL**

**of 22 September 2003**

**on additives for use in animal nutrition**

**(Text with EEA relevance)**

**(OJ L 268, 18.10.2003, p. 29)**

Commission Regulation (EC) No 386/2009 of 12 May 2009 L 118 66 13.5.2009

*ANNEX I*

**ADDITIVE GROUPS**

(m) substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action.

**A mycotoxin binder (adsorbent/denaturant) added to the diet should be able to interact *in vivo* with mycotoxins and restrict their absorption in the GI tract of animals**

## **Several requirements for a mycotoxin binder**

- Effective inactivation of mycotoxins of interest
- Reduction of mycotoxin bioavailability/activity
- Availability of essential nutrients to an animal
- Not being detrimental to animal or food product
- Not being a growth promoter (growth promotion may mask mycotoxins)
- Demonstrate verifiable positive results (improvement of zootechnical performance; recovery of organ status; excretion of mycotoxins via faeces; recovery of the immune status)
- Technical and economical feasibility

## My first contact with “binders”...

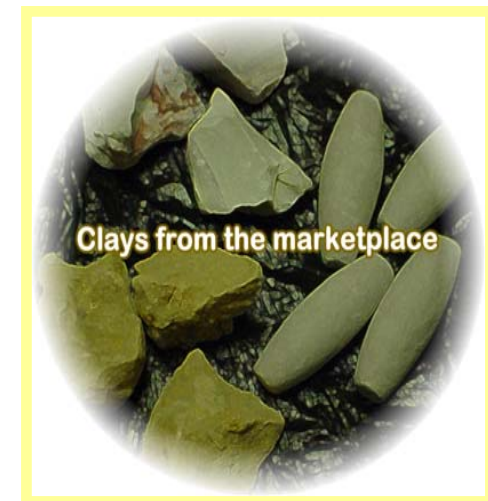
First, macaw and parrot clay licks are special deposits of clay along riverbanks or sometimes in the forest interior in the Amazon rainforest. The birds flock to the clay, usually in waves that start at 600 or 630 am and end at 1100 am or noon, to eat thumb-sized lumps each day. The clay appears to detoxify the nasty poisons in their diets of seeds of rainforest trees and vines.

<http://www.inkanatura.com/macawclaylicks.asp>



Munn, C.A., 1994. Macaws: winged rainbows. Natl. Geograph. 185:118-140.

TD Phillips is working with Don Brightsmith, Head of Rainforest Expeditions, to determine the reason for geophagy in Macaws in Peru.



Humans in Ghana

## ■ Adsorbents

### ■ Inorganic

- Activated charcoal
- Aluminosilicates, clays

HSCAS, bentonite, montmorillonite, zeolite, clinoptilolite...

Clay minerals – layered silicates  $[\text{Si}_2\text{O}_5^{2-}]_{xy}$

Zeolite – silicate made of tetrahedrons of  $\text{SiO}_4$  and  $\text{AlO}_4$

- Chemically treated silicates

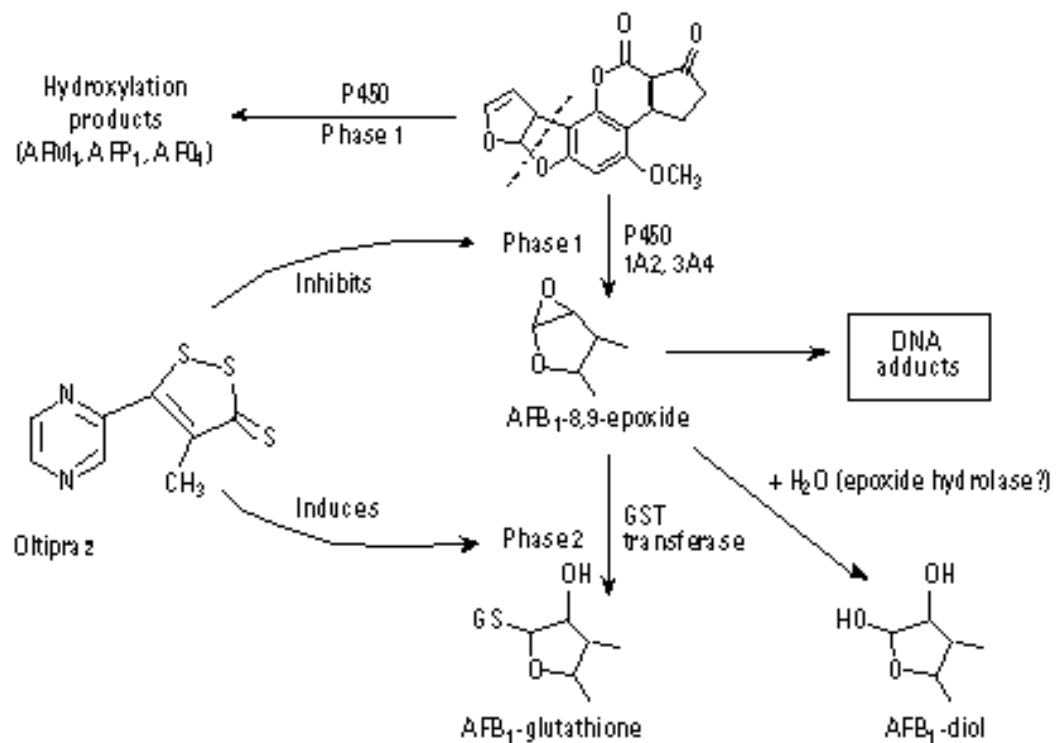
### ■ Organic

- Chemical polymers (cholestyramine, polyvinylpyrrolidone, divinylbenzene-styrene polymers...) High costs
- Glucan products

Yeast and products from yeast (yeast cell wall)

## Denaturant

- Mycotoxin-degrading enzymes
- Life microorganisms producing mycotoxin-degrading enzymes
  - *Eubacterium sp.* (BBSH 797)
  - *T. mycotoxinivorans*



**Animal health (in vivo)**

**Metabolic indicators (in vivo)**

**Cell culture studies (in vitro)**

**Isotherm studies (analysis of suspensions)**

**Effects on analysis????**

## **Cascade of steps in analytical methods for mycotoxins:**

- Extraction**
- Clean-up / Dilution / Concentration
- Separation
- Detection

### Official Methods (or those in standardization process):

- Aflatoxin B1 → EN ISO 17375:2006
- Deoxynivalenol → prEN 15791:2009
- Zearalenone → prEN 15792:2009
- Ochratoxin A → WD N954 of TC327/WG1
- Fumonisin → WD N953 of TC327/WG1
- T2 and HT2 toxins → Breidbach et.al. under consideration as a prospective CEN standard.

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<b>Mycotoxin</b>	<b>Average RSD<sub>r</sub> (%)</b>
DON	10.6
AFB1	6.9
ZEA	7.7
T2 & HT2 toxins	9.8
FUM B1 + B2	3.2
OTA	4.1

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Code	Type of main product(s)	pH value	Amount added [%]
1	Yeast cell wall	2.9	0.2
2	Clay + organic acid	7.9	0.1
3	Clay	8.4	0.1
4	Clay	4.6	0.25
5	Yeast cell wall	6.2	0.2
6	Yeast cell wall	6.7	0.2
7	Yeast cell wall	4.7	0.25
8	Mixture organic + mineral	6.2	0.25
9	Yeast cell wall	5.0	0.2
10	Mixture organic + mineral component	8.6	0.25
11	Mixture organic + mineral component	6.9	0.25
12	Clay	9.1	0.5
13	Clay	8.9	1
14	Fibres	6.7	1
15	Montmorillonite	10.4	0.4
16	Montmorillonite	10.6	0.4
17	HSCAS	8.8	0.5
18	HSCAS	9.8	0.5
19	HSCAS	9.8	0.5
20	Fibres (lignocellulose)	5.1	2.5

COMMISSION DIRECTIVE 2003/100/EC  
of 31 October 2003  
amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on  
undesirable substances in animal feed  
(Text with EEA relevance)

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
(1)	(2)	(3)
7. Aflatoxin B1	All feed materials	0,02
	Complete feedingstuffs for cattle, sheep and goats with the exception of:	0,02

COMMISSION RECOMMENDATION

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

(Text with EEA relevance)

(2006/576/EC)

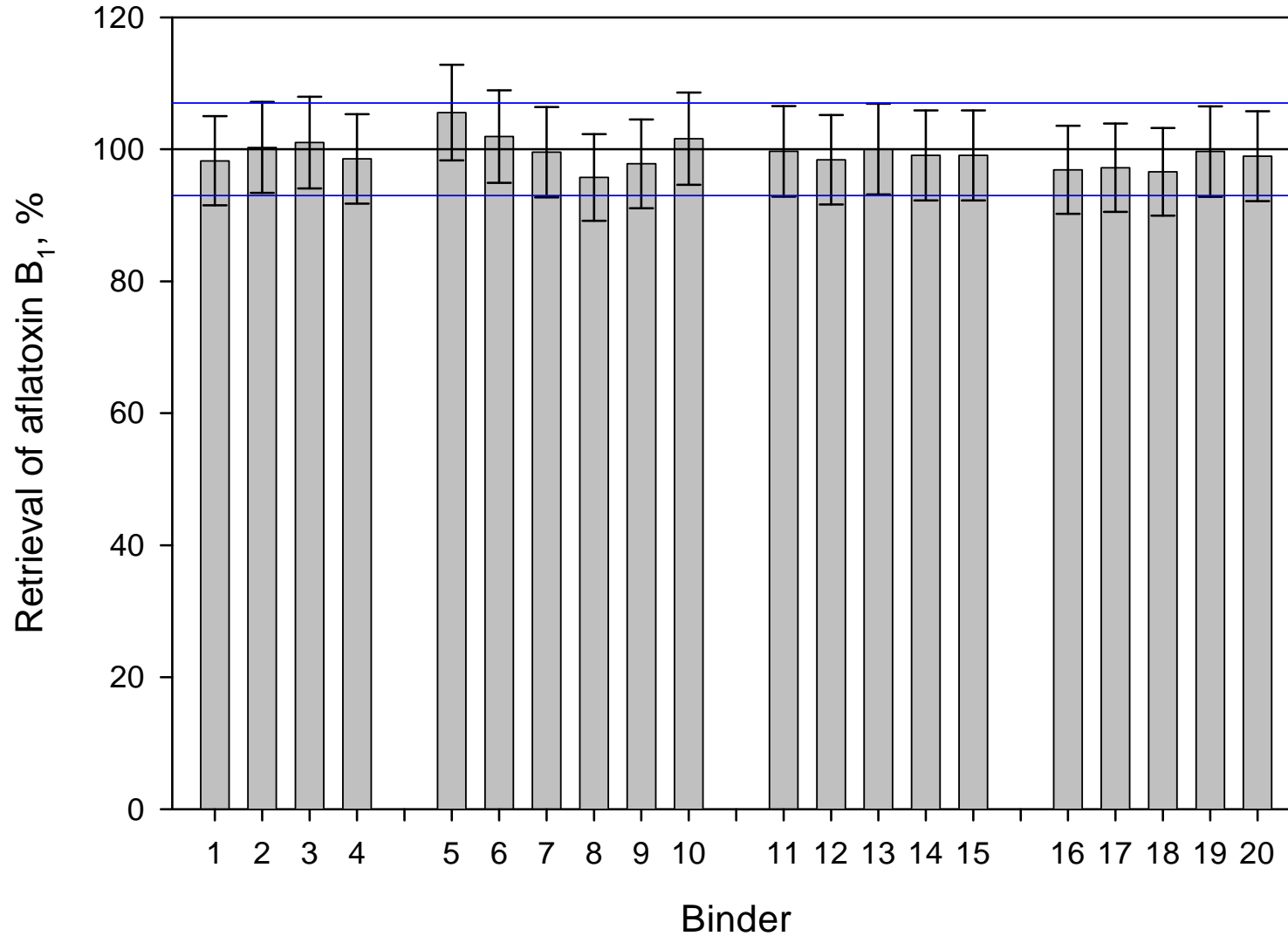
Mycotoxin	Products intended for animal feed	Guidance value in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 %
Deoxynivalenol	Feed materials (*)	
	— Cereals and cereal products (**) with the exception of maize by-products	8
	— Maize by-products	12
Zearalenone	Feed materials (*)	
	— Cereals and cereal products (**) with the exception of maize by-products	2
	— Maize by-products	3
Ochratoxin A	Feed materials (*)	
	— Cereals and cereal products (**)	0,25

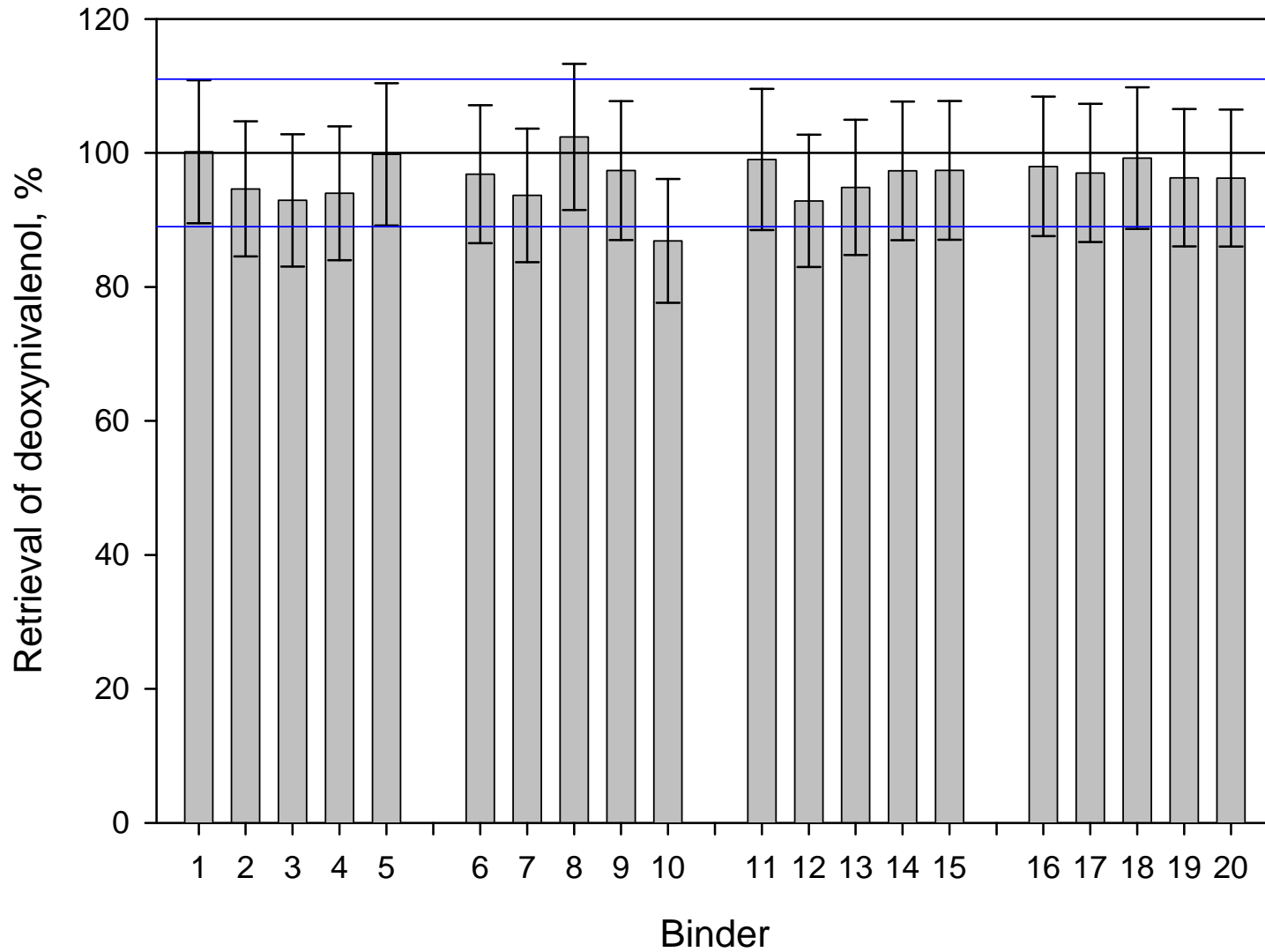
## Data analysis

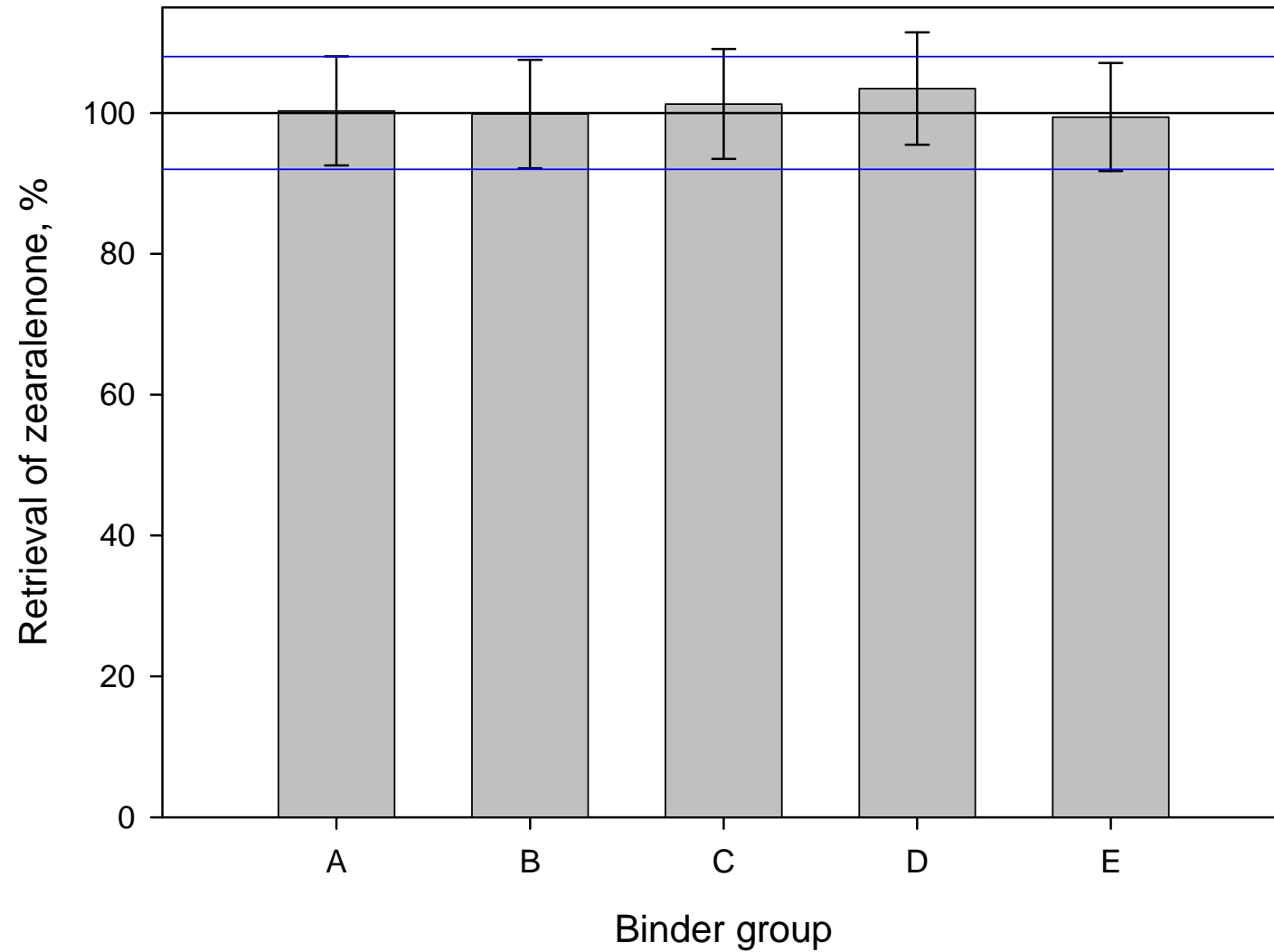
Student's *t*-test (independent two-sample *t*-test with unequal sample sizes and equal variance). Differences were assumed to be significant at  $p < 0.05$ .

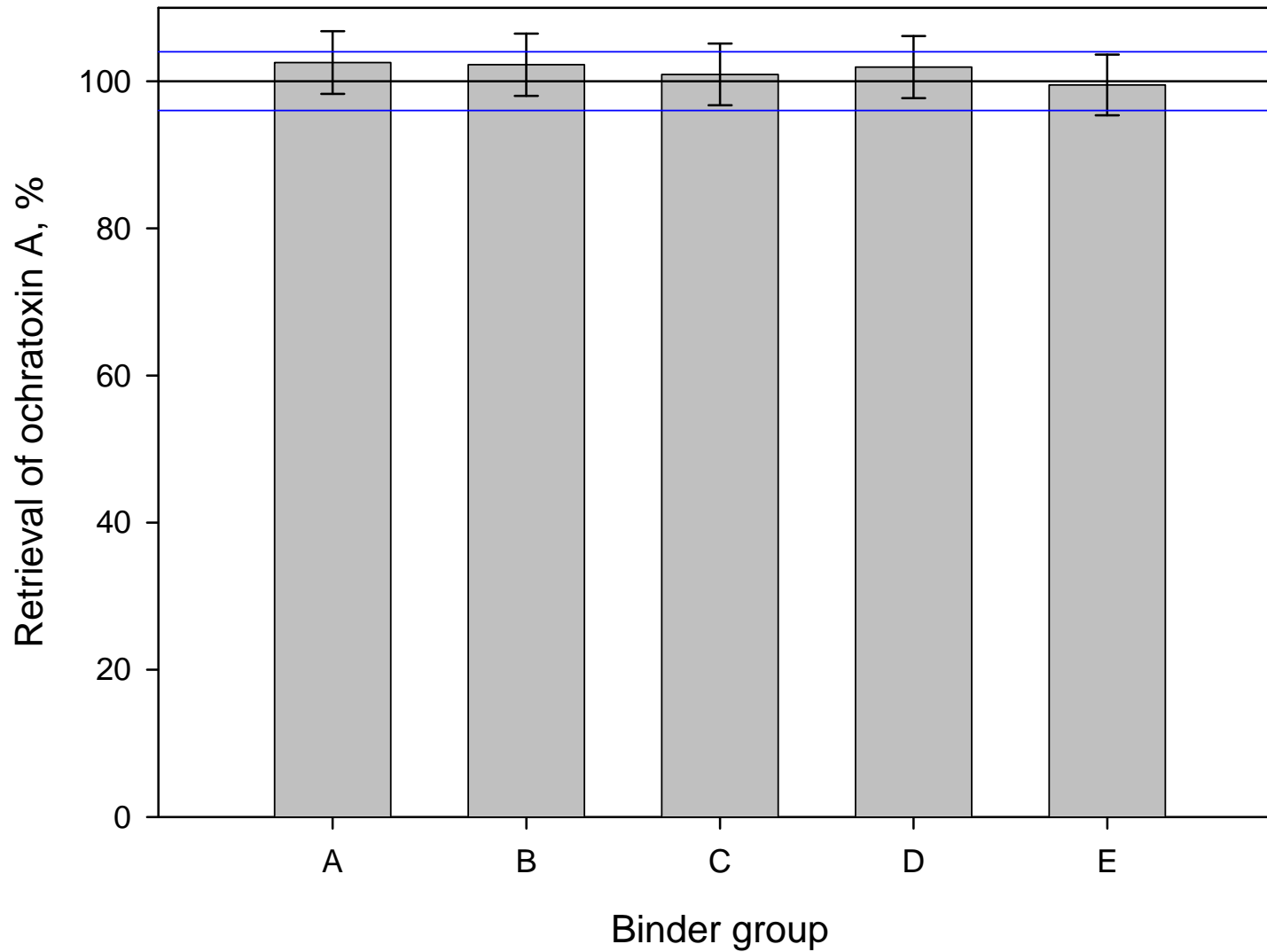
Critical *t*-values ( $p = 0.05$ ) were compared with those obtained by the Student's *t*-test according to the following formula:

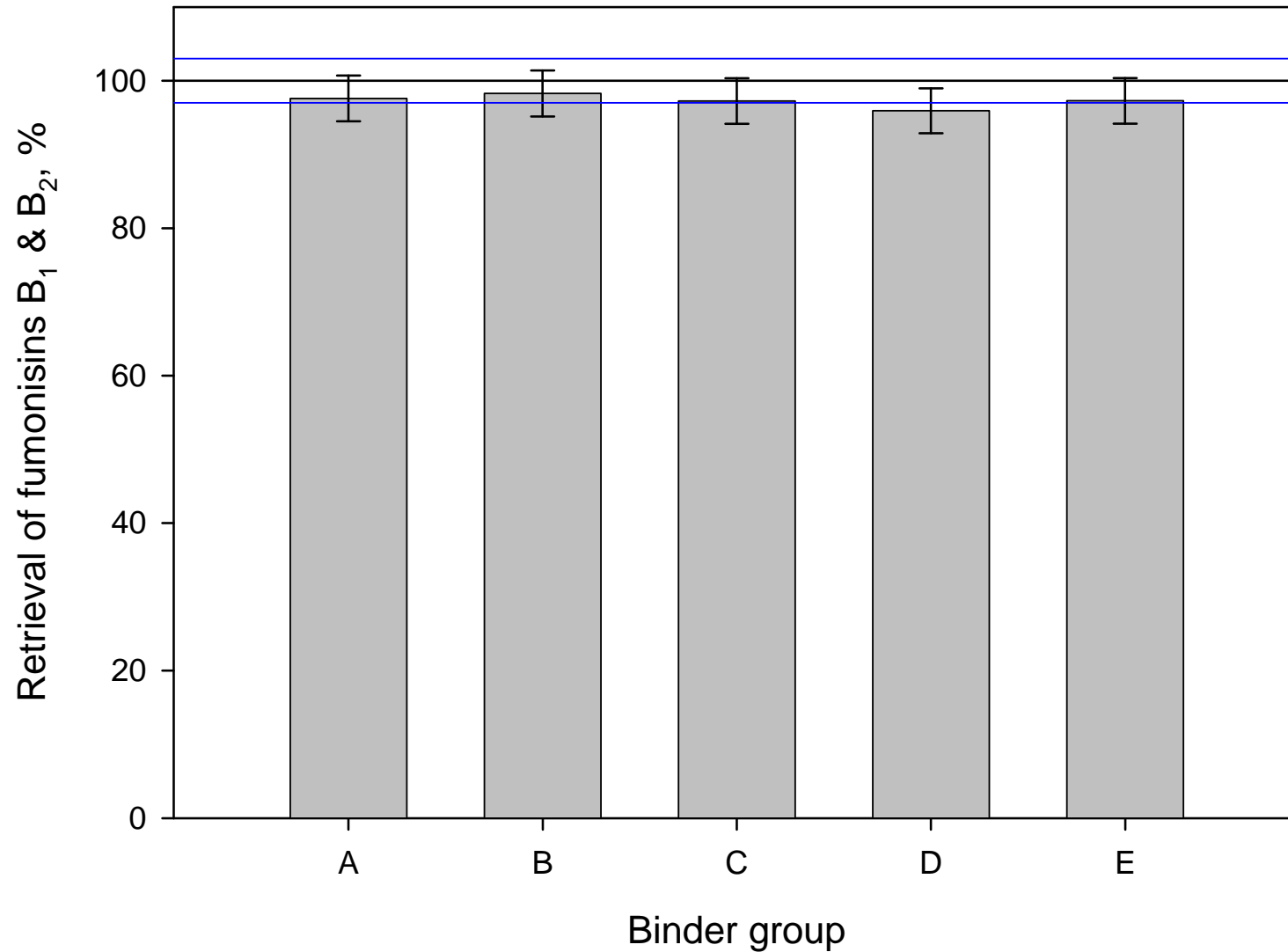
$$t = \frac{\bar{X}_1 - \bar{X}_2}{S \times \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

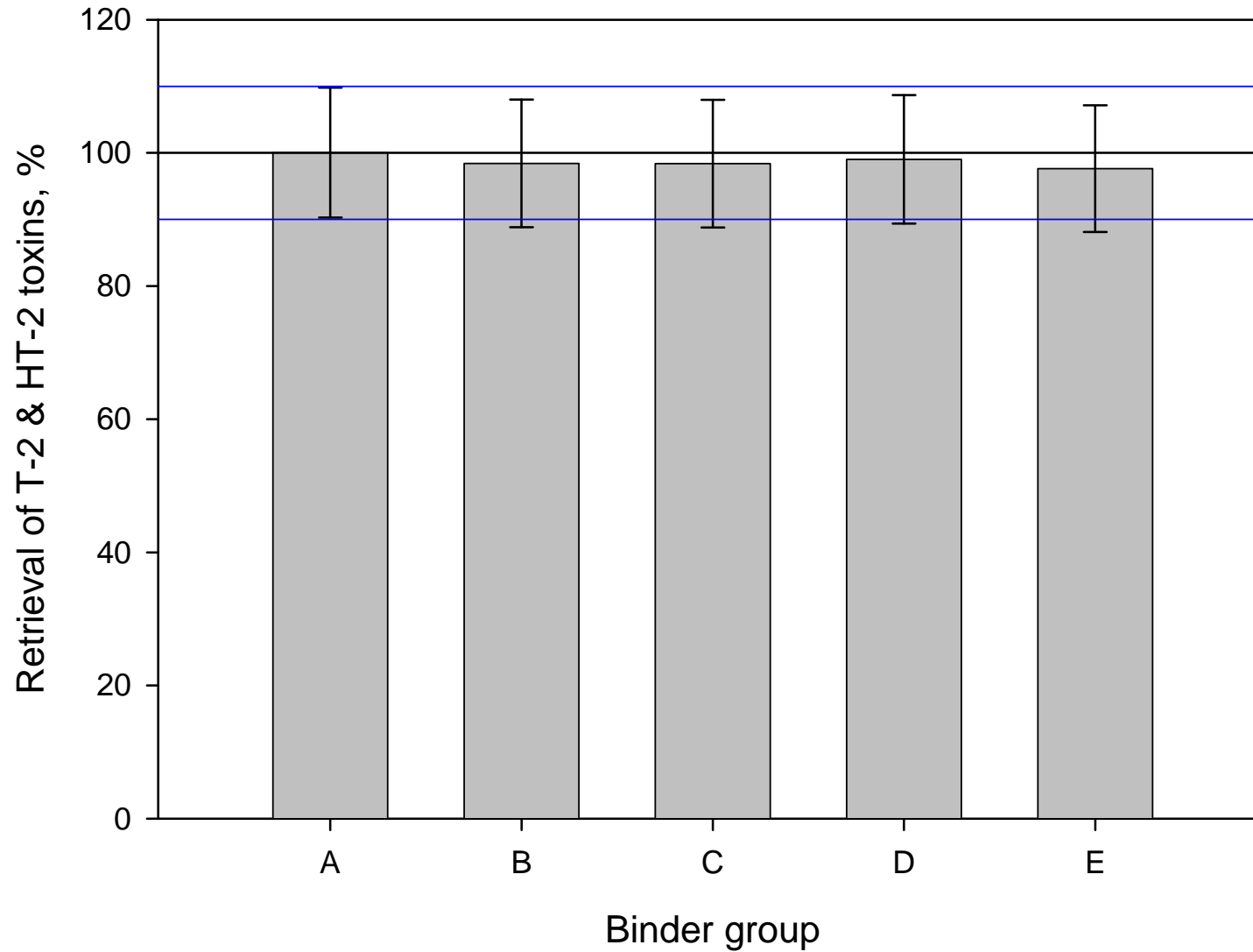












## Ouissam Abbas and Vincent Baeten In collaboration with **Alexandro Rodriguez**

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Quality of Agricultural Products Department

**Chaussée de Namur, 24**

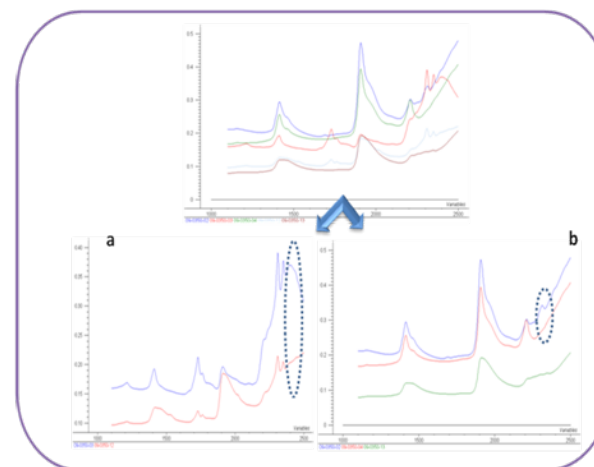
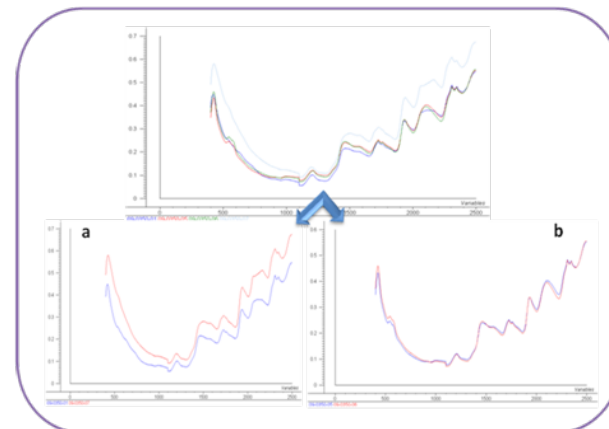
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## Effect of the inclusion of adsorbents on aflatoxin B<sub>1</sub> quantification in animal feedstuffs

A. Gallo\*, F. Masoero, T. Bertuzzi, G. Piva and A. Pietri

*Istituto di Scienze degli Alimenti e della Nutrizione, Facoltà di Agraria, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, I-29100 Piacenza, Italy*

*(Received 6 May 2008; final version received 21 July 2009)*

The extraction efficiency of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in cattle feed containing nine adsorbents (ADSs) was investigated using two organic/aqueous solvents composed of methanol/water (80/20 v/v; MeOH) and acetone/water (85/15 v/v; AC).

Means were compared with the correspondent controls using the Dunnett's test. No statistical difference was found in AFB<sub>1</sub> levels of feedstuffs not containing ADSs when extracted with AC or MeOH, even if numerically higher values were obtained with AC.

Consequently, a daily AFB<sub>1</sub> consumption of 123.7 or 23.3 µg per cow per day can be calculated, and a milk contamination of 0.149 or 0.030 µg kg<sup>-1</sup> of AFM<sub>1</sub> can be predicted. The latter value, calculated on the basis of the MeOH result, is well below the limit of 0.050 µg kg<sup>-1</sup> set by the European Commission (2006), but the real situation is quite different, as reported in our previous work (Masoero et al. 2009).

## Assessment of deoxynivalenol (DON) adsorbents and characterisation of their efficacy using complementary *in vitro* tests

S. Cavret\*, N. Laurent, B. Videmann, M. Mazallon and S. Lecoeur

*Umr Inra-Dger-Isara-Lyon, Métabolisme et Toxicologie Comparée des Xénobiotiques, AGRAPOLE, 23 rue Jean Baldassini, 69364 Lyon cedex 7, France and Ecole Nationale Vétérinaire de Lyon, 1 av. Bourgelat, BP. 83, F-69280 Marcy l'Etoile, France*

*(Received 30 April 2008; final version received 28 April 2008)*

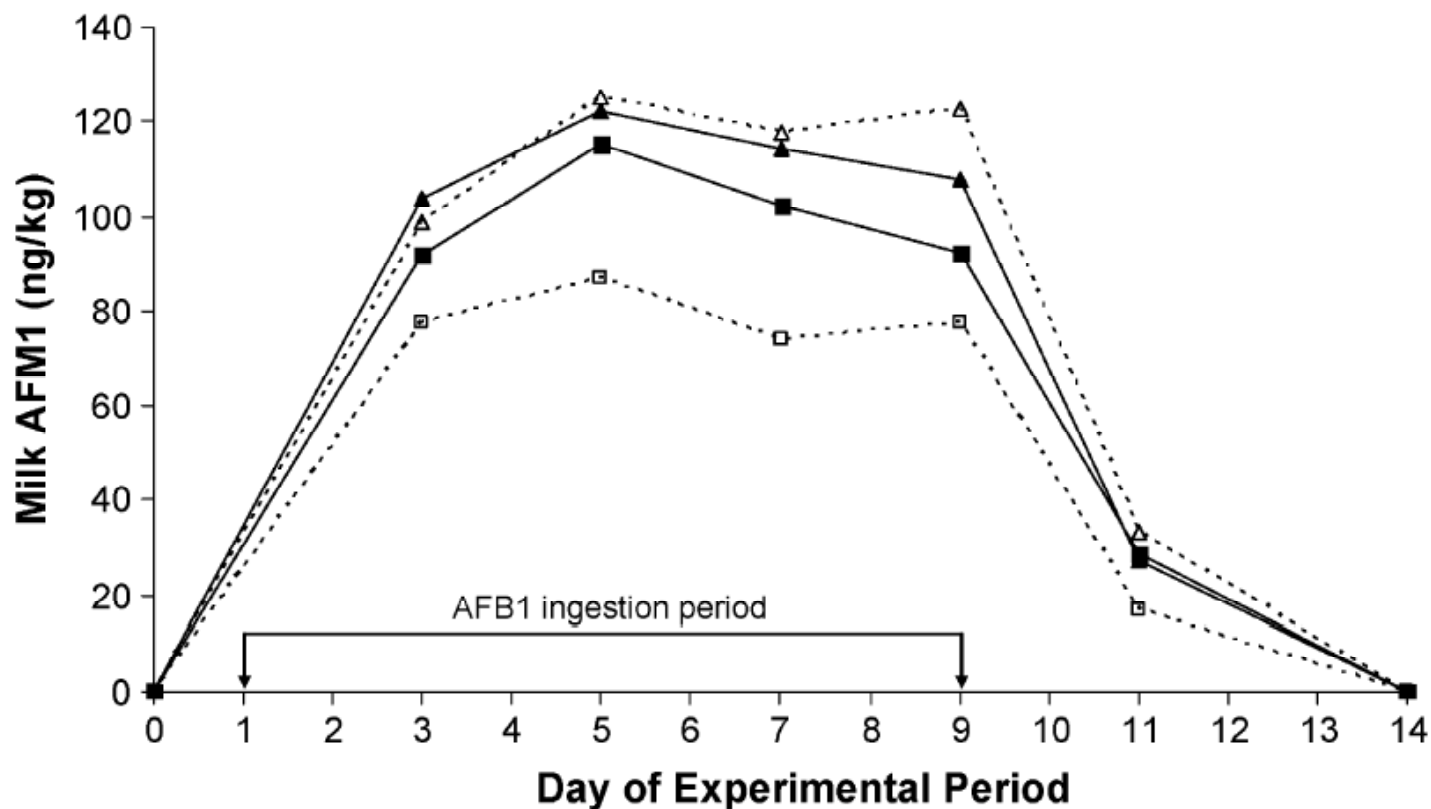
Charcoal did not significantly bind DON when not activated.

Our results supported the hypothesis that DON adsorption by activated charcoal would be of ~90% for DON concentrations lower than 2 mg/g and where the pH was between 3 and 8

Our

study confirmed that *Saccharomyces cerevisiae* man-  
nans could bind DON and specified that DON adsorp-  
tion rate decreased as DON concentration increased.

In contrast, pH showed itself to be a determinant factor – the rate of DON adsorbed decreasing with pH. Some material, such as cholestyramin or leguminous plant, no longer bound DON at pH 3.



**Fig. 1.** AFM1 concentration (ng/kg) in milk of cows fed different diets (experiment 2): (■) CC-SA, (□) Pellet-SA, (▲) Meal-SA, and (△) PMX-SA. For each diet, the point represents the mean of eight values (treatment from days 1 to 9 included). Treatment (excluding days 0, 11 and 14), day and treatment×day interaction *P* values: <0.001, <0.001 and 0.450, respectively (S.E. = 3.39).

**The addition of the tested binders (at the level indicated by the manufacturer) did not result in lower analytical values for any mycotoxin currently regulated in feed.**

**The combined effect of processing such as wet heating, pH-change, etc. in the presence of mycotoxin binders was not investigated, but might be a subject of interest for other studies.**



**Massimo Ambrosio**  
**Katy Kroeger**  
**Katrien Bouten**

## Poster # 22:



**The influence of mycotoxin binders on the performance and validity of an LC-MSMS multi-mycotoxin method.**

Theo de Rijk<sup>a,b</sup>, Ed Boers<sup>a</sup>, Paul Zomer<sup>a</sup>, Hans Mol<sup>a</sup>, Jörg Stroka<sup>c</sup>

## IRMM is looking for collaborators for projects on:

characterisation studies  
homogeneity and stability studies, and  
feasibility studies



As well as for scientific staff (e.g. post docs)

[www.irmm.jrc.be/html/calls/call\\_for\\_collaborators/index.htm](http://www.irmm.jrc.be/html/calls/call_for_collaborators/index.htm)

[www.irmm.jrc.be/html/job\\_opportunities/index.htm](http://www.irmm.jrc.be/html/job_opportunities/index.htm)