



**1st MoniQA International Conference:
Increasing trust in rapid analysis for food safety**
Rome, Italy from October 8-10, 2008

EU Community / National Reference Laboratory structure – harmonisation of food control

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(State Institute for Chemical and Veterinary Analysis of Food)



Community Reference Laboratory (CRL) for dioxins and PCBs in feed and food

CRL for pesticides in food of animal origin and commodities with high fat content

WHO Reference Laboratory for dioxins, PCBs and halogenated pesticides (POPs) in human milk

Organisation of CVUA Freiburg

1	2	3	4	5	6	7
GC	Water (1)	Pesticides (1) *)	Wine	Diet food	Meat (1)	Pathology
GC-MS, HPLC-MS	Water (2)	Pesticides (2) *)	Liquors, beer	Genetic. mod. Food	Meat (2)	Bacterio- logy
HPLC	Environm. Contam.	Vet. Drugs (1)	Juices	Oils, fat	Fish	Virology
Heavy metals	Consumer goods	Vet. Drugs (2)	Honey, sweets	Vegetables	Milk (1)	Serology
Radio isotopes	cosmetics	Dioxins *)		Molecular biology	Milk (2)	Bees diseases
Toxicology					Micro- biology	Fish diseases
					Immuno- logy	Poultry diseases

*) CRL

CRL: Community Reference Laboratory



Community Reference Laboratory
for dioxins and PCBs in feed and food



Community Reference Laboratory
for pesticides in food of animal origin
and commodities with high fat content

Selection of CRLs

- Call of interest in all Member States (July 2005)
- Submission of comprehensive dossiers for evaluation
- National Selection until October 2005
- Evaluation at EU: EFSA (European Food Safety Authority) and FVO (Food and Veterinary Office)
- Decision in January 2006

Since 1 Jan 2007: 27 EU Member States



Freiburg

General provisions (1)

Regulation (EC) No 882/2004 of the European Parliament
and of the Council of 29 April 2004
**on official controls performed to ensure the verification
of compliance with feed and food law,
animal health and animal welfare rules**

General provisions (2)

Three levels of laboratories according to Reg. 882/2004:

- Art. 12:
Official laboratories (OFLs)
- Art. 33:
National Reference Laboratories (NRLs)
- Art. 32:
Community Reference Laboratories (CRL)

CRL- Reg. 882/2004

Article 32 - CRL



The CRL shall be responsible for

- Providing NRLs with details of **analytical methods, including reference methods**
- organising **comparative testing** and by ensuring an appropriate follow-up of such comparative testing
- Conducting initial and further **training courses** for the benefit of staff from national reference laboratories
- Providing scientific and technical **assistance to the Commission**, especially in cases where Member States contest the results of analysis

CRL- Reg. 882/2004

Article 33 – NRLs



Member States designate one or more national reference laboratories (NRLs) for each Community laboratory. These NRLs shall

- Collaborate with CRL
- Co-ordinate for their area of competence the activities of official laboratories
- Organise where appropriate comparative tests between the official national laboratories and ensure follow-up of such comparative testing
- Ensure the dissemination to the competent authority and official national laboratories of information that the CRL supplies

Regulation for new CRLs

COMMISSION REGULATION (EC) No 776/2006

of 23 May 2006

amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community reference laboratories

- CRLs for feed and food
(21 sectors)
- CRLs for animal health and life animals
(13 sectors)

CRLs (Com Reg 776/2006) - 1

CRLs for feed and food:

- Milk and milk products,
- Proteins in feedingstuffs,
- Additives for use in animal origin
- Material intended to come into contact with foodstuffs
- marine biotoxines,
- Genetically modified organism (GMO)
- Antimicrobial resistance,
- ...

CRLs (Com Reg 776/2006) - 2

CRLs for feed and food (veterinary parameters)

- Transmissible spongiform encephalopathies (Tsex),
- Zoonoses,
- Viral and bacteriological contamination of bivalve molluscs,
- *Listeria monocytogenes*,
- *Staphylococcus aureus*,
- *E. coli*,
- *Campylobacter*,
- Parasites,

List of CRLs for veterinary drugs (Reg 776/2006)

12. Community reference laboratories for residues of veterinary medicines and contaminants in food of animal origin

- (a) For the residues listed in Annex I, Group A 1, 2, 3, 4, Group B 2(d) and Group B 3(d) to Directive 96/23/EC

Rijksinstituut voor Volksgezondheid en Milieu (RIVM)
3720 BA Bilthoven
The Netherlands

- (b) For the residues listed in Annex I, Group B 1 and B 3(e) to Directive 96/23/EC and carbadox and olaquinox

Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants
AFSSA — site de Fougères
BP 90203
France

- (c) For the residues listed in Annex I, Group A 5 and Group B 2(a), (b), (e) to Directive 96/23/EC

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)
D-12277 Berlin
Germany

- (d) For the residues listed in Annex I, Group B 3(c) to Directive 96/23/EC

Instituto Superiore di Sanità
I-00161 Roma
Italy

List of pesticide CRLs (Reg 776/2006)

17. Community reference laboratories for residues of pesticides

(a) Cereals and feedingstuffs

Danmarks Fødevareforskning (DFVF)
DK-1790 København V
Denmark

(b) Food of animal origin and commodities with high fat content

Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg
Postfach 100462
D-79123 Freiburg
Germany

(c) Fruits and vegetables, including commodities with high water and high acid content

Laboratorio Agrario de la Generalitat Valenciana (LAGV)
Grupo de Residuos de Plaguicidas de la Universidad de Almería (PRRG)
LAGV: E-46100 Burjassot-Valencia
PRRG: E-04120 Almería
Spain

(d) Single residue methods

Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart
Postfach 1206
D-70702 Fellbach
Germany

List of CRLs for other residues (Reg 776/2006)

18. Community reference laboratory for heavy metals in feed and food

The Joint Research Centre of the European Commission
Geel
Belgium

19. Community reference laboratory for Mycotoxins

The Joint Research Centre of the European Commission
Geel
Belgium

20. Community reference laboratory for Polycyclic Aromatic Hydrocarbons (PAH)

The Joint Research Centre of the European Commission
Geel
Belgium

21. Community reference laboratory for dioxins and PCBs in feed and food

Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg
Postfach 100462
D-79123 Freiburg
Germany



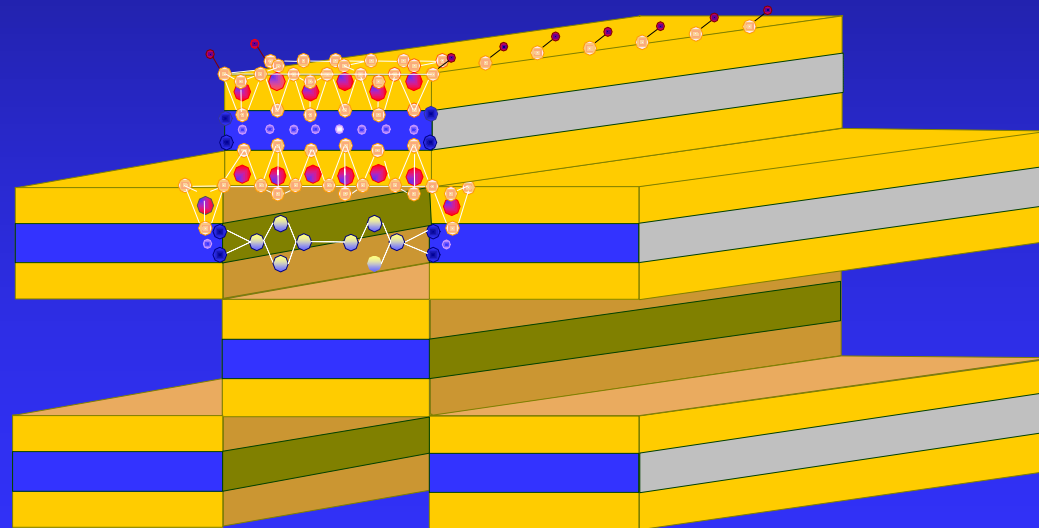
Harmonization of analytical methods

Contribution of the Community Reference Laboratory to Standardize Methods

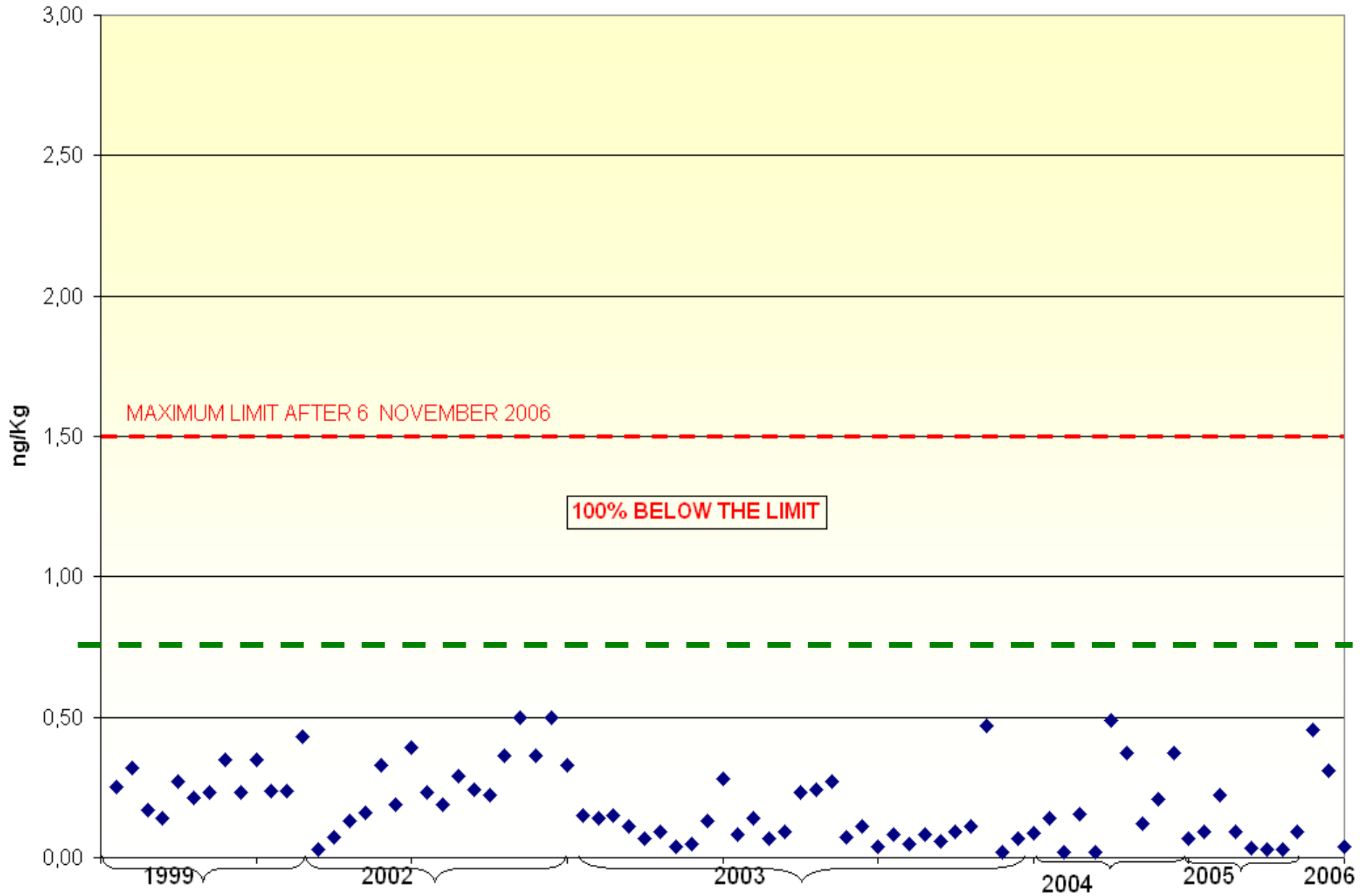
Harmonization of Extraction Methods for the Determination of Dioxins in Mineral Feed

Sepiolite as a binder and anti-caking agent

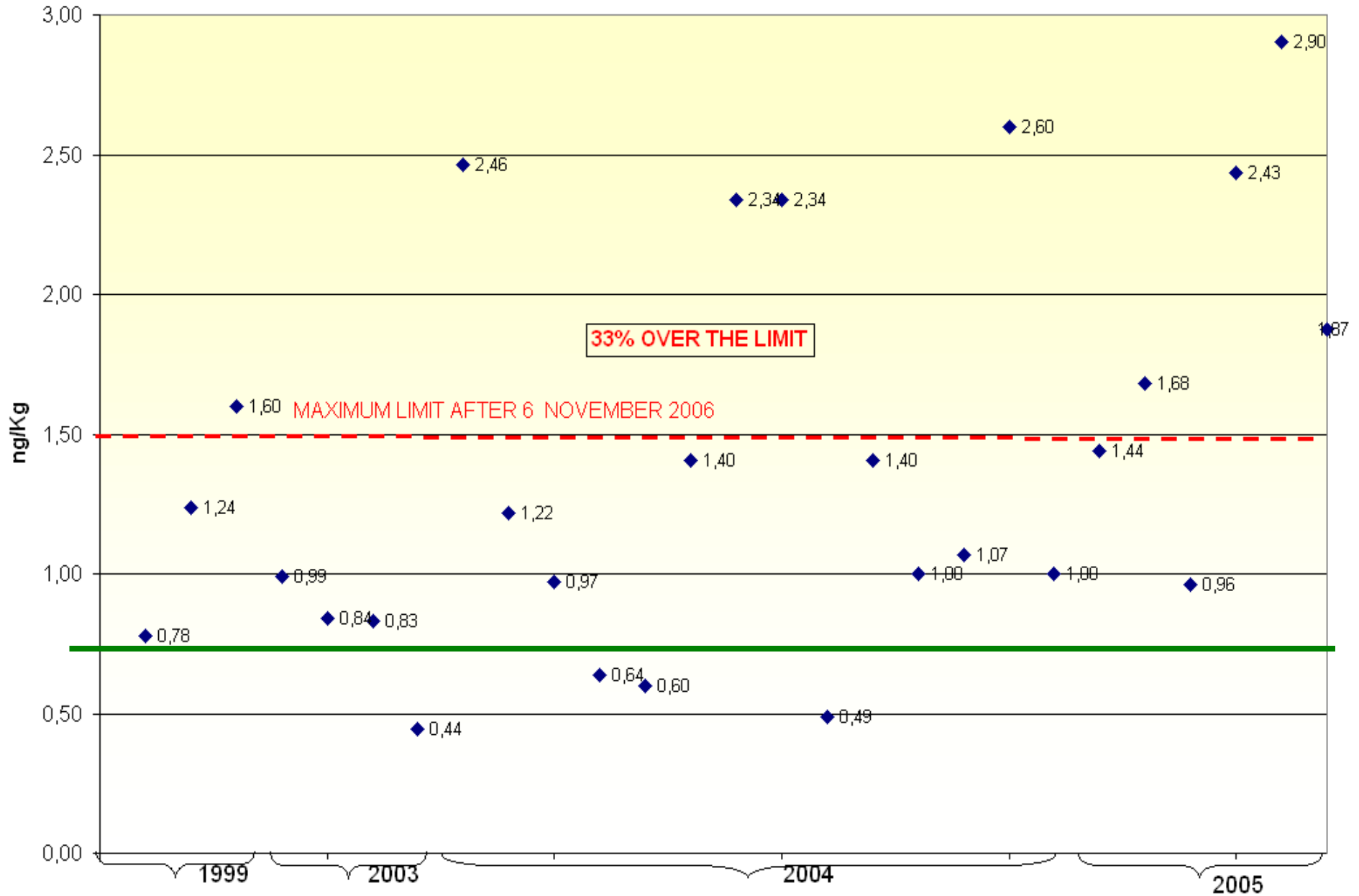
- Sepiolite is a clay belonging to the family of Phyllosilicates, which is being used in animal nutrition as additive E-562.
- It is present in the EU market since more than 20 years and more than 2 million tons already consumed



SOLVENT EXTRACTION: DIOXINS & FURANS IN SEPIOLITE



ACID EXTRACTION: DIOXINS & FURANS IN SEPIOLITE





CRL-Workshop Freiburg, October 12, 2006

Extraction method for dioxins in feed with high mineral content

Comprehensive study on dioxin content in sepiolite

-

Influence of different parameters on the extraction of sepiolite

**Alexander Kotz and Rainer Malisch
CRL for Dioxins and PCBs in Feed and Food
Freiburg**

Parameters for interlaboratory study



- ✓ Test material: Sepiolite
- ✓ Extraction techniques:
 - ◆ Toluene (Soxhlet),
 - ◆ Other routine extraction methods
 - ◆ Digestion with 3 n HCl before extraction



- ✓ Analysis of a sepiolite extract, reagent blanks and an analyte solution

Participating laboratories

- ✓ **17 laboratories** (including CRL Freiburg) participated in the study
- ✓ **15 laboratories** reported results until October 6, 2006

Summarizing conclusion (2006)

- Toluene + polar solvent (e.g. ethanol, acetone, isopropanol) best suitable extraction medium
- Substantial fraction of all solvents required

Study in 2007

- **Manganese Oxide (MnO)**

Used as additive in feedingstuff



- **Fullers Earth (mineral clay)**

Can also be used for food contact
(e.g. bleaching earth)





CRL-Workshop Freiburg, November 5-6, 2007
Extraction methods for dioxins in feed with high mineral content

Determination of dioxins in mineral feed, premixtures and compound feed

Summary:
Conclusions for extraction

Summarizing conclusions

1. For **mineral feeds such as clays (except oxides of trace elements) and compound feeds**, a mixture of toluene and polar solvents (e.g. ethanol, acetone, isopropanol; in substantial proportion) is the best extraction medium.
2. For **oxides of trace elements**, toluene is the most suitable solvent.
3. There is no need to pretreat with HCl.
4. For an **unknown premixture or premixture with problematic composition**, it is recommended to combine two extraction steps, e.g. first with toluene and then with a toluene / polar solvent mixture.

Consequences of this standardisation

- Recommendations will be binding for official food and feed control in EU Member States
- Practically, this will become binding for private laboratories analyzing food and feed samples for producers or trade in EU Member States, as well
- Consequently, this will effect international trade

Contamination
by pentachlorophenol (PCP)
and dioxins
of guar gum
originating from India

PRODUCTION OF GUAR GUM

- Guar gum powder is extracted from the guar bean
- India produces about 80% of the world's total production of guar beans
- World market in the range of 100 000 tons per year
- Food and non-food use

THE CONTAMINATION INCIDENT

Very high contamination levels of dioxins and pentachlorophenol (PCP) found in certain batches of guar gum:

- about 1000 times the level of what can be considered as normal background contamination

Regulatory levels for pentachlorophenol

- ✓ MRL of 0.01 mg/kg for PCP for all food and feed foreseen in a draft Commission Regulation amending Regulation 396/2005



Levels of **PCP** exceeding **0.01 mg/kg** (taking into account the measurement uncertainty) are to be considered unacceptable

Regulatory levels for PCDD/F

- ✓ No maximum levels established for guar gum in Commission Regulation (EC) No. 1881/2006
- ✓ With reference to other regulations:



Levels of **WHO-PCDD/F-TEQ** exceeding **0.75 pg/g** (taking into account the measurement uncertainty) are to be considered unacceptable

Underestimation of PCP levels (results of self-control of industry)

Reliability of analytical results for PCP questionable

Guar gum batch no. 11560:

Results	PCP (mg/kg)	WHO-PCDD/F-TEQ (pg/g)
CRLs Stuttgart/Freiburg	33.4	457
other laboratory	4	406

- Considerable underestimation of PCP level
- Possible problem: insufficient extraction of PCP from matrix?

Possible reasons for underestimation of PCP

Experiences of labs of CRR/NRL/OFL network :

1. CVUA Münster, Peter Fürst:

Extraction with acidified acetonitrile compared to extraction with organic solvent caused a 10fold increase of the PCP content in a sample

2. CRL Stuttgart, Hubert Zipper / Michelangelo Anastassiades:

water content at extraction and pH value important for correct PCP determinations

➤ Need for harmonization of analytical methods in particular for PCP

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- Need for harmonization of analytical methods in particular for PCP
- EU mission to India found finally : Na-PCP used in India. This confirms proposed analytical aspects (water –soluble; pH dependent)



Community Reference Laboratory
for Dioxins and PCBs in Feed and Food



State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany

Chemisches und Veterinäruntersuchungsamt Freiburg
PO Box 100462 • D-79123 Freiburg • Germany

in cooperation with



Chemisches und Veterinäruntersuchungsamt Stuttgart, PO BOX 1206, 70702 Fellbach, Germany

Announcement
of a proficiency test (PT) on determination of
dioxins (PCDD/F), PCBs (dioxin-like PCBs and indicator PCBs)
and pentachlorophenol (PCP)
in guar gum samples

Overview of Analytical Results for Determination of Dioxins and PCP in Guar Gum

- Need to Harmonize Analytical Methods -

Community Reference Laboratory for Dioxins and PCBs in Feed and Food
(State Institute for Chemical and Veterinary Analysis of Food)
Freiburg, Germany

Kerstin Wahl, Rainer Malisch, Johannes Hädrich, Alexander Kotz

Conclusions

„Overview of Analytical Results for Determination of Dioxins and PCP in Guar Gum - Need to Harmonize Analytical Methods”

Analysis:

- ✓ Reliability of PCP results in some cases questionable – especially in the low concentration range

- ✓ Need for analytical methods able to determine reliably
 - PCP in the range of 0.01 mg/kg
 - Dioxins in the range of 0.75 pg WHO-PCDD/F-TEQ/g

Proficiency test



REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 23 February 2005

on maximum residue levels of pesticides in or on food and feed of plant and animal origin and
amending Council Directive 91/414/EEC

Article 28

Methods of analysis

1. The methods of analysis of pesticide residues shall comply with the criteria set out in the relevant provisions of Community law relating to official controls for food and feed.

2. Technical guidelines dealing with the specific validation criteria and quality control procedures in relation to methods of analysis for the determination of pesticide residues may be adopted in accordance with the procedure referred to in Article 45(2).

3. All laboratories analysing samples for the official controls on pesticide residues shall participate in the Community proficiency tests for pesticide residues organised by the Commission.

**Pesticide Residues:
Mandatory
participation
of NRLs and OFLs
at CRL-PTs (1)**

Pesticide Residues: Mandatory participation of NRLs and OFLs at CRL-PTs (2)

- NRLs are responsible to name all relevant OFLs of the respective MS.
- Possible cooperation for participation of OFLs through NRLs: provision of data for country-specific evaluation to NRLs

Proficiency Test “Pesticides in food of animal origin, 2006”

Summary: coverage of analytes

Organochlorines		Organophosphorus		Pyrethroids	
Pesticide	% covered	Pesticide	% covered	Pesticide	% covered
Aldrin	100	Chlorpyriphos (-ethyl)	75	Deltamethrin	75
alpha-HCH	100	Chlorpyriphos-methyl	75	Permethrin (sum)	70
Dieldrin	100	Diazinon	75	Cypermethrin	65
Endrin	100	Pirimiphos-methyl	70	lambda-Cyhalothrin	65
p,p'-DDD	100	Methacrifos	30	Bifenthrin	55
p,p'-DDE	100	Fenamiphos	25	Fenvalerate	50
p,p'-DDT	100	Formothion	15	Cyfluthrin	40
beta-HCH	95				
gamma-HCH	95				
Heptachlor	95				
o,p'-DDT	95				
alpha-Endosulfan	90				100 % of the labs
HCB	90				75 % up to 100 % of the labs
alpha-Chlordane	75				50 % up to 75 % of the labs
beta-Endosulfan	75				25 % up to 50 % of the labs
gamma-Chlordane	75				less than 25 % of the labs
trans-Heptachlorepoxid	75				
Oxychlordane	70				
cis-Heptachlorepoxid	65				
o,p'-DDD	65				
o,p'-DDE	65				
4,4'-Methoxychlor	60				
Endosulfan sulphate	55				
Nitrofen	40				

Conclusion of first PT (2006) “pesticides in food of animal origin”

- In general, three groups of pesticides can be considered to have been introduced in daily routine of NRLs for pesticides in animal food and high fat commodities: **organochlorine, organophosphorous and pyrethroid pesticides**
- **Room for improvement of qualitative (number of analytes) and quantitative (deviation of results from consensus value) aspects**

Standardized Methods

vs.

Methods validated within a single laboratory

General provisions for methods of analysis

(Regulation 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules)

(Art 11) Sampling and analysis methods used in the context of official controls shall comply with relevant Community rules or

- if none exist, internationally recognised rules or protocols, for example those that the European Committee for standardisation (CEN) has accepted or those agreed in national legislation; or
- In the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols

In case the above mentioned does not apply, validation of methods of analysis may take place within a single laboratory according to an internationally accepted protocol.

Availability of standardized methods



AOAC, DIN / CEN, country-specific methods (e.g. official collection according to § 35 LMBG (old) - § 64 LFBG (new))

- For many parameters (food additives, residues of pesticides or drugs used in veterinary medicine, contaminants): **standardized methods ("official methods")**
- If official methods are available and applied: Lab has to show that it can apply these methods successfully.

Steps for development of “official” methods (approach for veterinary drugs in Germany)

Methods have to be described in detail and to be followed in all details

1. **validated (intra-laboratory, method developer)**
2. **peer-verified (e.g. in 3 other laboratories)**
3. **collaboratively tested (e.g. in >10 laboratories)**

Problem: Lack of analytical capacity

Complete methods vs. modular system

- Standardized methods for drug residue analysis (in Germany, only?): complete methods (including extraction, clean up and determination)
- Standardised methods for pesticide residue analysis (in Germany, only?): use of modular system allowing combination of single steps (extraction, clean up and determination)

Modular system for pesticide methods (1): extraction

European Standard	Description	German Standard	Modul	Description	Validated methods of CVUA Freiburg	Samples / Compounds
EN 1528-2: 1996-10 (confirmed 2001)	Extraction of fat, pesticides and PCBs and determination of fat content	§ 64 LFGB: L 00.00-34 (confirmed 1999)	E 8	Extraction of fat with hexan/acetone	PV 31 P01601	meat, fish
	Extraction of fat, pesticides and PCBs and determination of fat content	§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.1	Extraction of milk	PV 31 P00402 PV 31 P00502	milk
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.2.3	Extraction of butter	SOP 31 S00303	butter
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.3.1 and 6.3.2	Extraction of cheese, dairy and milk powder	PV 31 P00202 PV 31 P00302 PV 31 P00602	cheese, dairy, milk powder
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.4	Extraction of meat, fisch and products	PV 31 P00202 PV 31 P00302 PV 31 P01601	meat, fish and products
		§ 64 LFGB: L 00.00-38/2 (confirmed 09/1998)	6.5	Extraction of eggs	PV 31 P00202 PV 31 P00302 PV 31 P01601	egg
		§ 64 LFGB: L 01.00-8 (confirmed 04/1981)	-	Determination of the fat content of milk	PV 31 P00102	milk

Modular system for pesticide methods (2): clean up; detection

European Standard	Description	German Standard	Modul	Description	Validated methods of CVUA Freiburg	Samples / Compounds
EN 1528-3: 1996-10 (confirmed 2001)	Clean-up methods	§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	GPC	Gel permeation chromatography	PV 31 P00802	animal fats
		§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	C 2	Adsorption chromatography on a small silica gel column	PV 31 P00902	animal fats
		§ 64 LFGB: L 00.00-37 and 38/3 (confirmed 09/1998)	11	GPC and adsorption chromatography on a small silica gel column	PV 31 P00802 PV 31 P00902 PV 31 P01002	animal fats
		§ 64 LFGB: L 00.00-12 (confirmed 08/1993)	7.4.2	Clean-up with sulfuric acid	PV 31 P01102	animal fats
EN 1528-4: 1996-10 (confirmed 2001)	Determination, confirmatory tests, Miscellaneous	§ 64 LFGB: L 00.00-38/4 (confirmed 09/1998)	4 - 5	Gas chromatography with ECD, FPD, NPD, MSD	PV 31 P00802 PV 31 P00902 PV 31 P01002	organochlorine -, organophosphorus-, sulfur-containing-, nitrogen-containing-compounds, pyrethroids and other
		§ 64 LFGB: L 00.00-34 (confirmed 11/1999)	D1 - D4			
	GC analysis of organotin compounds in fish and mussels	§ 64 LFGB: L 10.00-9 (confirmed 12/2002)	-	organotin compounds are transferred into ethyl-compounds and detected by GC-FPD and GC-MS	PV 31 P01401	fish, mussels

Standardised methods for pesticides

- Some reliable, approved and over long time tested methods
- New methods being developed (in particular with LC/MS and QuEChERS / different modifications)

Conclusions from residue analysis (1)

- ✓ A lot of efforts to support and sustain standardized methods
- ✓ Constant developments and changes caused by
 - Legal regulations (tolerances, MRPLs ...)
 - Technical progress (equipment)
 - Validation criteria

Conclusions from residue analysis (2)

- **Standardisation useful up to a certain degree, but flexibility necessary (criteria approach)**
- **Cooperation between CRLs and CEN for harmonization of methods**

Conclusions from residue analysis (3)

- Determination of analytes at trace levels in food and feed is a complex problem
- Specific knowledge of “food and feed chemistry” required
(biological samples different from environmental samples)
- Not only one „true“ analytical approach
- **Criteria approach for performance**
- **Complex quality control required**

Community Control Programme

CRL Workshop
with NRLs for Pesticides in Food of Animal Origin
and Commodities with High Fat Content

30 September 2008
Freiburg, Germany

Community Control Programme



- **Article 29 to Regulation (EC) 396/2005**
(entered into force 01. September 2008)

The Commission shall prepare a coordinated multiannual Community control programme

New: The programme should also cover food of animal origin

- **Criteria to be applied:**

- Monitoring samples shall be included in the national control programmes
- Taking into account problems of pesticide-product combinations that have been identified regarding compliance with the MRLs
- Serving the purpose of assessing consumer exposure of pesticide intake

Community Control Programme



- Draft Commission Regulation concerning a coordinated Community monitoring programme for 2009
 - As to the agreement of the MS, the monitoring shall cover **two different kinds of commodities for every year of a three-year cycle**
 - 6 representatives of the European diet are being discussed:
 - butter, egg, milk, ham, poultry meat and another commodity (cheese from sheep or goat?)
 - **Selection of mandatory and voluntary analytes under discussion**

General conclusions

- **CRL/ NRL network contributes to harmonization of food and feed control in various areas**