JRC TECHNICAL REPORTS

7th Workshop of the European Reference Laboratory for Heavy Metals in Feed and Food

Brussels, 20th September 2012

B. de la Calle, F. Cordeiro, I. Fiamegkos,
B. Kortsen, S. Roulette

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Joint Research Centre

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Joint Research Centre
Institute for Reference Materials and Measurements

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European Union Reference Laboratory

Heavy Metals in Feed and Food

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Minutes of the 7th Workshop of the European Union Reference Laboratory for Heavy Metals in Feed and Food

Brussels 20/09/2012

Welcome and opening of the event

The operating manger of the European Union Reference Laboratory for Heavy Metals in Feed and Food (EU-RL-HM), Beatriz de la Calle, opened the event welcoming the representatives of the 31 National Reference Laboratories (NRLs) attending the workshop this year. B. de la Calle also introduced the invited speaker, Daniel Tholen, who was responsible for the training offered during the workshop, which in this occasion dealt with the update on ISO 13528 (international standard dealing with the statistical evaluation of data coming out of proficiency tests (PTs)).

Update on European legislation on contaminants

B. de la Calle explained to the participants that this presentation had been cancelled because the speaker, Almut Bitterhof, could not attend the workshop due the problem of methanol in spirits in the Czech Republic, which had caused already several casualties in the Czech Republic and some of its neighbour countries.
B. de la Calle said that no big changes have taken place in the European legislation on contaminants since the last workshop. The updates of that legislation as presented in the last workshop by A. Bitterhof follow the normal administrative procedure, and accordingly it can still take same time till their final implementation.
B. de la Calle informed the NRLs that the EU-RL-HM had been requested by the Directorate General for Health and Consumers (DG SANCO) to compare the mass fraction of lead obtained in kaolinitic clay feed samples after total digestion and after partial extraction with 5 % HNO_3 at boiling temperature for 30 min, according to Directive 2002/32/EC on undesirable substances in animal feed. The reason for this request was that according to the producers of kaolinitc clay as feed, the two mass fractions obtained with the two methods described above are significantly different. The analysis performed by the EU-RL-HM confirmed the statements of the producers, as summarised in the report that was submitted to DG SANCO (JRC 62122). DG SANCO will decide how to tackle this issue when the footnote on partial extraction will be removed from the legislation as it is foreseen.

Update of the EU-RL-HM activities during the last 12 months

B. de la Calle presented to the participants the activities carried out by the network since the last workshop (held on 22nd September 2011), which includes a PT (IMEP-114) on heavy metals in feed pre-mixes and a collaborative trial for the validation of a method to determine methylmercury in food of marine origin (IMEP-115). B. de la Calle said that as part of the EU-RL-HM duties, she had attended the meetings of the CEN TC 275/WG 10 (Trace Elements and their Species in food) and CEN TC 327/WG 4 (Trace Elements and Minerals in Feed). The former working group will standardise a method for the determination of aluminium in food. Joachim Engman from the Swedish NRL for food made a presentation on a specific problem encountered in Sweden related to the determination of Al in noodles. Significantly different results were obtained for the mass fraction of Al in noodles in presence

or absence of HF during the sample digestion. The two laboratories involved in the dispute on results are accredited and used a validated method (the validation included bias determination of the method with use of a certified reference material, CRM). The CRM used to validate the method which does not include the use of HF, was wheat flour. No bias could be detected during the validation. The problem seems to be restricted to noodles, where the Al has been fraudulently added (Al as additive is forbidden in the European legislation for food additives). A possible explanation is that Al could have been added in the form of silicates and so it would require the addition of HF for a quantitative recovery.
Gerhard Liftinger, from the Austrian NRL, said that they analyse around 60 samples per year, digesting the samples with HNO_3 in open digestion systems, using ICP-AES, to avoid dilution steps. He mentioned that contamination is always a problem in the analysis of Al.
Nine NRLs perform Al analysis on a regular basis.
Marina Patriarca, from the Italian NRL for food, said that she had worked in the past in the field of Al determination and that Al presents few problems from a toxicological point of view because t is effectively eliminated from the body via the kidneys, and for that reason it only represents a problem for people with kidney failure undergoing dialysis. This opinion is in agreement with that expressed by the experts in Al analysis contacted by B. de la Calle last year, when trying to find an expert to give a presentation on Al determination in food, at the 6^{th} Workshop organised by the EU-RL-HM. Those experts indicated that from a toxicological point of view the determination of Al is only of relevance in biological fluids but not in food. For this reason, the question remains weather it is the task or not of this network to address the issue of Al determination in food commodities.
In the frame of the activities carried out by the CEN TC 327/WG 4, Jens Sloth, from the Danish NRL for food, informed the participants that a new standard method, EN 16278, has been published for the determination of inorganic arsenic (iAs) in feed. The method developed by the Danish NRL for Food (Technical University of Denmark) is based on the selective separation of iAs by solid phase extraction (SPE) cartridges and further determination by AAS. The method was validated in a collaborative trial, whose organisation was organised by DTU and the EU-RL-HM.
B. de la Calle informed the participants that with the appointment of the Institute of Public Health Maribor as Slovenian NRL for food of non-animal origin, the Danish Veterinary and Food Administration as Danish NRL for Feed and LabNett and Norwegian Veterinary Insitute as two new Norwegian NRLs, the network of the EU-RL-HM is now integrated by 50 members.
G. Liftinger said that for the matrices under the responsibility of the EU-RL-HM, AGES-Linz will be the Austrian NRL. AGES-Vienna will be the Austrian NRL for the matrices covered by the mandate of the European Union Reference Laboratory for Chemical Elements in Food of Animal Origin hold by the Istituto Superiore di Sanità.

Discussion about future PTs and trainings
Going through the e-mails submitted by the NRLs when asked about their preferences for PTs for 2014 (the EU-RL-HM work program for 2014 has to be ready by the end of August 2013, before the next workshop will take place), it became evident that most NRLs would like to have a PT for determination of heavy metals, including tin, in food of vegetable origin. The Italian NRL for food expressed its interest in having a PT on a fresh matrix (as consumed) and not in a lyophilised matrix, so that the test item would be a matrix as those with which laboratories are normally confronted. In this way it will also be possible to check how certain correction factors have to be implemented and taken into consideration, for instance, in the uncertainty calculation (another request of the NRL for food). To cover all the mentioned

subjects it was decided to organise in 2014 a PT for the determination of trace elements (including Sn) in canned food of vegetable origin.
In relation to the question of Sn determination, the representative of one of the Greek NRLs said that if the maximum levels for tin in food refer to inorganic Sn how should the speciation problem be solved. In canned food of marine origin a certain percentage of Sn will be present in the form of organic compounds. It was agreed that in those cases the mass fraction of organic compounds of tin should be determined and subtracted from the total content of Sn. The difference would give the mass fraction of inorganic Sn. This approach would represent a problem for most of the control labs in the food sector because most of them would not have methods in place for the determination of organotin compounds. Determination of organotin compounds is normally performed by environmental laboratories, because those compounds are covered by the Water Framework Directive. At the moment 12 NRLs determine Sn on a regular basis. Determination of total content of tin seems to be the more generalised approach. B. de la Calle will raise this issue to A. Bitterhof who is responsible for the updates of the European Legislation on contaminants in food.
Regarding the training to be offered during the next workshop, it was agreed to have several presentations on the new features of ICP-MS instrumentation. B. de la Calle said that to avoid problems with non-giving equal opportunities to all commercial manufactures of ICP-MS instruments, she will try to find one or several re-known researchers expert in the field of ICP-MS.

Training on the update of ISO 13528
Daniel Tholen, convener of the Technical Committee dealing with the revision of ISO 13528 (Statistical methods for use in proficiency testing by interlaboratory comparisons), made a presentation about the main changes that will be implemented in the new version of the mentioned standard which will replace the actual version in 2013 or 2014.
In the discussion that took place after the presentation several matters were addressed but they referred mainly to the issue of $\hat{\sigma}$ (standard deviation for proficiency assessment). In the new revision $\hat{\sigma}$ will, very likely, be called "standard error for proficiency assessment". Some of the participants considered inappropriate such a name because it refers to the distance between the assigned value and the individual result reported by the participants, while "standard deviation" refers to a dispersion of results around the assigned value.
B. de la Calle said that some NRLs indicate in their feedback to the PTs organised by the EU-RL-HM that the modified Horwitz equation should be used by default to calculate $\hat{\sigma}$. This approach is not regularly applied by the EU-RL-HM who tends to select $\hat{\sigma}$ taking into consideration the state-of-the-art in a particular field. NRLs are supposed to perform better than the normal population of control laboratories in Europe. D. Tholen supported this opinion; W. Horwitz said to him that it was not his purpose when developing that equation (further modified by M. Thompson) to produce a figure that could be used as $\hat{\sigma}$ and that such a use of the mentioned equation was not to be recommended.
M. Patriarca said that U_f (described in Regulation (EC) No 333/2007 as the maximum standard measurement uncertainty characterising methods used in official controls), should be used in the PTs organised on determination of the contaminants covered by that legislation (Pb, Cd, Hg and Sn in the case of heavy metals). J. Engman said that U_f only applies when analyses are performed using "in-house" validated methods but not when standardised methods are applied.
Paul Lawrance from the UK NRL for feed stressed the need to harmonise the different international documents dealing with statistical treatment of data coming out of PTs, for instance ISO 13528 and the IUPAC Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories.

Eight of the attending NRLs organise proficiency test in their respective countries.

The representative of the Italian NRL for food asked if it would be possible to find a modality with which the EU-RL-HM could support the NRLs in the organisation of PTs for the official control laboratories (OCLs). B. de la Calle indicated that in the last years the EU-RL-HM has allowed the NRLs to appoint OCLs to take part in the PTs organised by the EU-RL-HM in parallel to the PTs for the NRLs, using the same test item. In that case the appointing NRL pays the registration of the OCL. The EU-RL-HM must receive a written authorisation by the OCL to disclose their identity to the appointing NRL at the end of the exercise.
Some NRLs do not have a budget for the NRL activities and just announce the PT in the network of OCLs in their respective countries. In this case the participating OCLs pay their own registration and their results are not disclosed by the EU-RL-HM to the NRL. It is of course up to the NRL to negotiate with the OCLs the access to the scores obtained by a certain OCL.

Information on the outcome of the competitive project CONffIDENCE
J. Sloth made a presentation on the competitive project CONffIDENCE which main objective was to validate methods for the determination of organic and inorganic contaminants in food and feed. J. Sloth was responsible for the work package 3 dealing with development of methods to determine trace elements. In particular, two methods were developed for the determination of iAs and methylmercury, respectively, in food. Detailed information on both methods can be found in the presentation hand-outs included in this report.

Discussion of the outcome of IMEP-114/36 and IMEP-115
In the afternoon preliminary results of IMEP-114 (PT for NRLs for heavy metals in feed pre-mixes) and IMEP-36 (PT run using the same test item than IMEP-114 for all laboratories that wanted to register) were presented by Ioannis Fiamegkos, the newest member of the EU-RL-HM team. Only the preliminary results were presented because the assigned value to be used to score the results submitted by participants were not yet available.
Preliminary results of IMEP-115 (a collaborative trial to validate a method for the determination of methylmercury in food of marine origin) were presented by F. Cordeiro who was the coordinator of this ILC.

After the mentioned two presentations three discussion groups were organised:
1) Discussion of the outcome of IMEP-114 (chaired by I. Fiamegkos)
2) Discussion of the outcome of IMEP-115 (chaired by F. Cordeiro)
3) Discussion about the significant figures for the measurement result and its uncertainty (chaired by M. Patriarca).

Participants took part in the discussion that interested them more and some of them moved among the different working groups. A summary of what was discussed in the three groups was presented by each of the three chair-persons and is summarised here after:

Discussion of the outcome of IMEP-114
Participants agreed that feed-premixes are laborious matrices mainly regarding the determination of Sn. The members of the group agreed that tin should be included again in a forthcoming PT. The analysis of mercury was also demanding, because it was observed that when ICP-MS was used higher values were obtained compared to other techniques. The pre-treatment method used was also very important. Another issue raised was that the moisture content of the sample was not reproducible enough. Some participants reported that after the

acid digestion insoluble mater was present in the sample interfering with the analysis. This observation was followed by a conversation on the advantages and disadvantages of using HF during the acid digestion.

Discussion of the outcome of IMEP-115:

Discussion about the significant figures for the measurement result and its uncertainty:
According to the **COMMISSION REGULATION (EC) No 333/2007,** D.1.1.: "The results shall be expressed in the same units and with the same number of significant figures as the maximum levels laid down in Regulation (EC) No 1881/2006." However, no guidance is given on the number of significant figures to be used for the uncertainty of the measurement result. The WG discussed the following example:

The maximum level for Pb in milk is stated as 0.020 mg/kg
The measurement result is 0.019 mg/kg and its uncertainty 0.0002 mg/kg
How should this result be expressed in the test report?

The participants, all of which have accredited methods for the determination of Pb in milk, reported their current practice:

View	**Expression of measurement result**
a) adjust the measurement result to the measurement uncertainty	0.0190 ± 0.0002 mg/kg
b) adjust the figures of the measurement uncertainty to match those of the measurement result	0.019 ± 0.001 mg/kg
c) maintain the significant figures for the measurement result as stated by CR 333/2007 and express the measurement uncertainty with the same number of figures	0.019 ± 0.000 mg/kg
d) maintain the significant figures for the measurement result as stated by CR 333/2007 and express the measurement uncertainty with one more figure	0.019 ± 0.0002 mg/kg

The following guidance from EA (EA-4/16 G:2003 "EA Guidelines for the expression of uncertainty in quantitative testing", par. 7.6) was considered:

"7.6 The number of decimal digits in a reported uncertainty should always reflect practical measurement capability. In view of the process for evaluating uncertainties, it is rarely justified to report more than two significant digits. Often a single significant digit is appropriate. Similarly, the numerical value of the result should be rounded so that the last decimal digit corresponds to the last digit of the uncertainty. The normal rules of rounding can be applied in both cases.
For example, if a result of 123.456 units is obtained, and an uncertainty of 2.27 units has resulted from the evaluation, the use of two significant decimal digits would give the rounded values 123.5 units ± 2.3 units".

The WG conclusion was that the measurement result and its uncertainty should be expressed as indicated in d), since this complies with the requirements of CR 333 and provides appropriate information on the laboratory measurement capability.

Before closing the event the representative of the Greek NRL presented a PT scheme organised that NRL (General Chemical State Laboratory) for the determination of Cr and Ni

in plant materials. Interested participants were invited to contact the PT provider for more information.

B. de la Calle closed the event thanking the participants for attending the workshop and wishing them all a good trip back home.

Geel 9/10/2012

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Food Safety and Quality

European Union Reference Laboratory
Heavy Metals in Feed and Food

7th EU-RL Heavy Metals Workshop

Thursday, 20/09/2012

Brussels, CCAB –2A

AGENDA

09:00-09:30	Welcome and opening of the event	M.B. de la Calle
09:30-10:15	• Presentation of the EU-RL Activities of the last 12 months • Presentation of the Work Programme 2013 • Discussion of Work Programme 2014	M.B. de la Calle
10:15-10:45	Aluminium in Noodles from China	Joakim Engman
10:45-11:15	Coffee break	
11:15-12:15	ISO 13528: Statistical treatment for use in proficiency testing by interlaboratory comparisons	D. Tholen
12:15-12:45	CONffIDENCE project	J.J. Sloth
12:45-14:00	Lunch	
14:00-15:50	• Presentation and discussion on the outcome of IMEP-114 and IMEP-115	F. Cordeiro I. Fiamegkos M.B. de la Calle
	Coffee break during poster presentation and discussions	
15:50-16:00	Closing of the event	M.B. de la Calle

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List of Participants		
Name	**Organisation**	**Country**
LIFTINGER Gerhard	Austrian Agency for Health and Food Safety	Austria
CHEYNS Karlien	Coda-Cerva	Belgium
GOSCINNY Séverine	Scientific Institute of Public Health	Belgium
WAEGENEERS Nadia	CODA-CERVA	Belgium
KIRILOVA Tsvetelina	Central Laboratory of Veterinary Control and Ecolo	Bulgaria
STEFANI Dimitris	State General Laboratory, Cyprus	Cyprus
SIMAKOVA Alena	State Veterinary Institute Olomouc,Lab.Kromeriz	Czech Republic
NIEDOBOVA Eva	CISTA	Czech Republic
ROKKJÆR Inge	Danish Veterinary and Food Administration	Denmark
SLOTH Jens	National Food Institute	Denmark
LILLEORG Roman	Veterinary and Food Laboratory	Estonia
VENÄLÄINEN Eija-Riitta	Evira	Finland
NOEL Laurent	ANSES	France
SOPHIE Rosset	Laboratoire SCL de Bordeaux	France
PALEOLOGOS Evangelos	General Chemical State Laboratory, Div of Ioannina	Greece
TABORHEGYI Eva	CAO-FFSD-Central Feed Invest. Lab	Hungary
DAVIDSON Frederick	Cork Public Analyst's Laboratory	Ireland
PASTORELLI Augusto Alberto	Istituto Superiore Sanità	Italy
PATRIARCA Marina	Istituto Superiore di Sanità	Italy
PAVLOVA Irina	Institute of food safety, Animal health and Enviro	Latvia
SATAITE Janina	NFVRAI	Lithuania
ZAMMIT Annabelle	Public Health Laboratory	Malta
LEE, VAN DER Martijn	RIKILT	Netherlands
NAWROCKA Agnieszka	National Veterinary Research Institute	Poland
STARSKA Krystyna	National Institute of Public Health	Poland
ASSIS TEIXEIRA Maria Gabriela	Laboratório Nacional de Investigação Veterinária	Portugal
CIOCILTEU Soniea	Hygiene and Veterinary Public Health	Romania
FARKAŠOVÁ Lívia	State veterinary and food institute - Košice	Slovakia
PAVŠIČ VRTAČ Katarina	National Veterinary Institute	Slovenia
MIRAT Manuela	Laboratorio Arbitral Agroalimentario	Spain
ENGMAN Joakim	National Food Administration	Sweden
BAXTER Malcolm	The Food and Environment Research Agency	United Kingdom
LAURANCE Paul	LGC Limited	United Kingdom
External Participants		
THOLEN Daniel	DanTholen Statistical Consulting	United States
FIAMEGKOS Ioannis	JRC IRMM	EC
DE LA CALLE Beatriz	JRC IRMM	EC
CORDEIRO RAPOSO Fernando	JRC IRMM	EC
KORTSEN Bibi	JRC IRMM	EC

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements

7th EU-RL Heavy Metals Workshop
CCAB Brussels, 20 September 2012
27 evaluations form received out of 34 participants

1. How would you rate the following information provided to you before the event?

	Excellent	Good	Fair	Poor	N/A*
Logistical information about the event (date, place, activities, program)	**19**	**8**	**0**	**0**	**0**
Information about the objectives and theme of the event	**11**	**14**	**2**	**0**	**0**
Information about the contents of sessions / presentations	**8**	**14**	**4**	**0**	**0**

* Not applicable

If poor indicate why:
- no information beyond the agenda

2. How would you rate the ...?

	Excellent	Good	Fair	Poor	N/A*
venue / facilities	**15**	**12**	**0**	**0**	**0**
catering / meals	**1**	**21**	**3**	**0**	**2**
registration procedure for the event	**15**	**12**	**0**	**0**	**0**
information provided during the event	**9**	**15**	**3**	**0**	**0**
assistance provided by JRC staff	**19**	**8**	**0**	**0**	**0**

* Not applicable

If poor indicate why:

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Telephone: direct line (32-14) 571 297.

3. How would you rate the ...?

	Excellent	Good	Fair	Poor	N/A*
length of the event	**7**	**19**	**1**	**0**	**0**
division of time between presentations and discussions	**8**	**19**	**0**	**0**	**0**

* Not applicable

If poor indicate why:

- I don't have a problem with one-day workshop too, I only have a feeling that then everything is inflated and overall is less topics which are discussed

4. Do you have any comments concerning the organisation of the event, or suggestions for improvement?

- I would like more time for exchanging opinions upon the presented issues, or other matters of concerns
- Personally I enjoyed the meetings in Geel more with two half days. I still have to spend one night away
- Maybe the event could be on two days so we would have time to discuss topics in the evening (as it was). Maybe in Brussels or in the national RL in different countries, so we could see the different labs and learn from each other.
- The event should have started at 9:00 as stated on the agenda (or as close as possible to that time) rather than at 9:30
- I very much appreciated the help and kindness of Ms Kortsen to enable my registration just few days before the meeting since it was my colleague Mr Auger who was due to come initially
- It would have been useful to circulate the IMEP results / questions prior to the event so that members could have brought the necessary experimental data for detailed discussion
- Good organization – no problems
- The inclusions of working groups provided a better opportunity for people to meet, to express their views on the topics and share experiences
- I found the organization ok
- No I was satisfied with the location and organization of the event
- When possible, the final agenda could be sent to us prior to the meeting
- The event was held in a well organized manner

5. Do you have any comments concerning the content of the event, or suggestions to improve events in the future?

- It would be nice to have a training course, taking place during the event, even though that could mean a second day
- More "practical" presentations about the trace analysis
- Discussions were good. Please continue with this concept. The lecture on PT statistics wasn't much focused. The actual statistical procedures were never covered. A lecture on PT could be more focused on the real life problems you will face in reality. Not just how to calculate statistics.
- Updated PowerPoint presentation handovers (notes) should have been made available during the workshop. Some speakers had changed their presentations and not amended their presentation notes.
- Due to the fact I have participated for the first time I have no comments. Maybe next time
- The item concerning ISO 13528 was not particularly relevant
- Good idea with the group discussion at the posters
- Beside the analytical issues it would be interesting if sometimes an updates on the health issues associated with heavy metals was included, e.g. on recent EFSA opinions
- I think that content of event is good
- I did find the accompanying printouts of a few presentations did not match the actual screen shots and was a bit thrown by this when trying to take notes and keep up with the presentation (I am easily thrown off course!)

6. To what extent do you agree with the following statements?

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
The event has improved my knowledge and expertise in my field of science and research	**7**	**16**	**4**	**0**	**0**

7. What is the name of the hotel you have selected and how would you rate it?

- Hotel du Parliament – a very good choice!
- Hotel Mozart – ***
- Holiday In Brussels Schuman, ok but expensive
- Best Western Park Premium Hotel
- Hotel Matignon, it is situated right in the centre – it was ok
- Silken Berlaymont – comfortable but expensive, short walk to CCAB
- Thon Hotel – very good
- Thon Brussels city center – good
- Hotel Des Colonies – just 3* hotel
- Hotel Plasky: good
- Best Western County House: correct
- NH Arenberg – good
- Thon Brussels City Center
- Floris Grand Place
- Hotel Plasky: good
- Hotel Floris Arlequin – it is ok
- Hotel Queen Anne- very good hotel
- Marti's Central Park hotel, it is normal and very close to event place
- Hotel Floris Arlequin Grand Place Hotel
- First Euroflat Hotel,
- Eurostars Sablon, typical functional business hotel, good central location about 20-30 minutes brisk walk from the venue. Could have used the underground for speed but prefer the exercise
- Hotel Mozart – it was nice looking and comfortable
- Thon Brussels city centre – good
- First flat hotel

8. Would you recommend it? Please indicate why / why not?

- (Hotel du Parlement) Yes I would. It is very close to Luxembourg station thus providing easy access to the airport and the city center, as well as the location where the meeting took place (even accessible on foot).
- (Hotel Mozart) I recommend the hotel because it is cheap and near the Grand Place.
- (Holiday In Brussels Schuman) Very near the CCAB that is an advantage.
- (Best Western Park Premium Hotel) I'll recommend this hotel. Nice area, not faraway from CCAB (20 min on foot), good meals.
- (Hotel Matignon) It was ok.
- (Silken Berlaymont) Yes, short walk to CCAB and close GO bus stop serving Brussels Airport.
- (Thon Hotel) Yes, good location, excellent service.
- (Thon Brussels city center) I would recommend. It is very near to the centre.
- (Hotel Des Colonies) Great location and with good price / value ratio.
- (Hotel Plasky) Close to the EU Institutions.
- (Best Western County House) The room was very clean and ok, the staff was nice, breakfast is included in the price but it is a bit noisy if the room is situated street side because of the bus traffic around the spot.
- (Thon Brussels city center) Yes, but a bit expensive (selected at short notice as part of Eurostar deal).
- (Floris Grand Place) Yes, ok standard, good location and fair price.
- (Hotel Plasky) Yes, comfortable, easy bus connection to CCAB (10 min), good and free of charge WiFi connection, reasonable price.
- (Hotel Floris Arlequin) Yes, the service was good and the room was comfortable. The hotel is well located.
- (Hotel Queen Anne) Yes, the hotel is central, close to train and metro stations, friendly staff, good breakfast and clean rooms.
- (Marti's Central Park hotel) Yes, I would do it, it is very close to event place.
- (Hotel Floris Arlequin Grand Place Hotel) No, I cannot recommend it – there was a lot of noise from the street during the night.
- (First Euroflat Hotel) It is very nice hotel, and the location is excellent.
- (Hotel Mozart) Feel free to recommend, simple way to the Commission buildings and close to the City centre.
- (Eurostars Sablon) the price has risen since my last visit, now probably borderline on cost. Still would recommend the hotel for a short stay.
- (Thon Brussels city centre) I would recommend it. It is very near to the centre.
- (First flat hotel) Good location.

Action: none

Distibution list to: F. Ulberth, B. De La Calle, B. Kortsen, D. Anderson, A. Cizek-Stroh, S. Roulette

Joint Research Centre

The European Commission's in-house science service

EU-RL-HM activities the last 12 months / Work Program 2013 / Discussion on Work Programme 2014 / And…

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Serving society
Stimulating innovation
Supporting legislation

Activities 2011 / 2012

- 6th Workshop of the EU-RL-HM network (21/09/2011)
- 14th PT on Total As, Cd, Pb, Hg and Sn in feed pre-mixes
- 15th ILC: collaborative trial for the validation of a method to determine methylmercury in food
- 7th Workshop of the EU-RL-HM network (20/09/2012) *(now)*
- Processing of a mushroom test item for the 16th PT to be held in 2013 is on-going

Activities 2011/2012

- Working Group of National Experts in Industrial and Environmental Contaminants
  Meetings attended on 10/10/2011 and 27/01/2012

- CEN/TC WG Trace Elements in food
  Meetings attended on 07/10/2011 (Stockholm) on 27/04/2012 (Berlin)
  Discussion about a suitable method for the determination of aluminium in food matrices
  Administrative work for validation of methods to determine iAs and methylmercury in food is *on-going*

- CEN/TC WG Contaminants, Minerals and Trace Elements in Feed
  Meeting attended on 24/05/2012 (Brussels)
  Standard for determination of iAs in feed to be published soon

News: some new members in the club, now we are 50! ☺

- SLOVENIA:
  Institute of Public Health Maribor *(food of non-animal origin)*

- DENMARK:
  The Danish Veterinary and Food Administration *(feed)*

- NORWAY:
  LabNett, avd. Stjørdal
  Norwegian Veterinary Insitute

Work Program 2013

- PT for the determination of Total As, Cd, Pb, Hg and of iAs and methylmercury in **mushrooms**

- PT for the determination of Heavy Metals in **compound feed**

- 8th Workshop (& training) (September 2013)

WP 2014

… your wishes for PTs (1):

- Cadmium in potatoes, Carrots, Parsnips, Cabbage etc.
- Aluminium in Flour, Bread, Infant Formulae, Infant Foods
- Tin in canned food such as fruit, vegetables, tomato puree.
- Cadmium and lead in bivalves or/and cephalopods
- Methylmercury in fish
- Cu in food and feed
- Pet feed
- Processed foods like ketchup, lasagne… or vegetables
- Food suplements, mostly made of plants (IMEP-106, SRM 3256)
- Tin in canned food





… your wishes for PTs (2):

- Vegetal matrices
- Closeness of PT samples to test samples (e.g. issues associated with the thawing of frozen foods; fresh/frozen materials vs freeze-dried ones or CRMs)



… your wishes for PTs (3):

- Ideas for PTs schemes
  - Mercury by ICP/AAS/MS
  - Arsenic by dry ash vs wet digestion
  - Lead and cadmium in feed
  - There is need for rapid PT's for emerging issues ???

… your wishes for trainings (1):

- Advances in sample preparation for analysis of metals by ICP-MS in order to increase sensitivity
- A review of ICP-MS techniques for Heavy Metals including improvements by instrument suppliers in order to minimise spectral interferences etc
- iAs speciation
- Determination of inorganic tin
- Contamination at low levels:handling the glassware and environment in the laboratory and cleaning of ICP MS ***(EN 13804 new revision soon available)***
- Processed and compound foodstuffs, with regard to the application of conversion factors and their effect on the measurement uncertainty and the assessment of compliance

… your wishes for trainings (2):

- How to avoid contaminations, impact of the high sensitivity of the new ICP-MS instrument on blank procedure results and how to handle isobaric interferences ***(see report of 5th Workshop organised by the EU-RL-HM)***
- Additional training in feed classification and categorisation and possibly tools for this purpose. Increased demand by EC 767/2009 and many feeds not properly categorised or labelled. Training/ development of methods appropriate for new formulations such as encapsulated vitamins, where existing procedures may not be appropriate.
- Sample preparation for difficult matrices for metals
- Digestion techniques for metals by ICP

… Problems you would like to discuss (1)

- This laboratory encountered matrix problems with the **IMEP-114** sample, which never happened before (i.e. **insoluble material** which required a change in the normal acid digestion procedure)
- I understand that the EU-RLs were formulating policy of **how to share such information with OCLs.** Is it possible to send reports from previous workshops to OCLs now?
- I also feel **more information on PT samples** is required to enable accurate execution of IMEP rounds e.g. IMEP111 was a mineral rock but was described as an 'animal feed' – these sample types require very different digestion conditions. Also, you are asking **measurement uncertainty estimates** in the IMEP-114 on as little as 2 or 3 replicates; this would involve the use of very large coverage factors if done properly. I am attending the meeting on the 20th so we can discuss further then
- We had general comments about the poor availability of animal feed reference materials especially for feed additives

… Problems you would like to discuss (2)

- Our laboratory has been experiencing problems with analysis of feed premix, in particular for Sn and Hg
- We had some problems with the determination of Hg and found in some samples with different methods (ICP-AES vs. ICP-MS vs. CV-AAS) very different concentrations of Hg

Discussion

Number of decimals when reporting uncertainties

COMMISSION REGULATION (EC) No 333/2007:

D.1.1 *"The results shall be expressed in the same units and with the same number of significant figures as the maximum levels laid down in Regulation (EC) No 1881/2006"*

D.1.3 *"The analytical result shall be reported as x +/– U whereby x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 % (U = 2u)"*

Eurachem QUAM: 2012.P1

Reporting uncertainty: *"Results should be rounded to be consistent with the uncertainty given"*

Number of decimals when reporting uncertainties

Maximum limit: 0.020 mg kg^{-1}

X= 0.019 mg kg-1
U= 0.0002 mg kg-1

How would you express the result??

Aluminium in Noodles from China

LIVSMEDELS VERKET
NATIONAL FOOD AGENCY

EU-RL HM in food and feed 2012-09-20
Joakim Engman

Control of imported Chinese Noodles

882/2004 Article 15:5

COMMISSION REGULATION (EC) No **669/2009**
'High-risk' products of non-animal origin

878/2010 Al in Chinese Noodles added

LIVSMEDELS VERKET
NATIONAL FOOD AGENCY

Al added as leavening agent?

- Limit: based on discussions on what's a normal level
  - **10 mg Al/kg (dry noodles)**
- That should prove addition of Al
- 10 % of imported consignments should be sampled

LIVSMEDELS VERKET
NATIONAL FOOD AGENCY

Swedish labs

- **Lab A, used by NFA for official control**
  - In-house
  - 0,3 - 0,5 g sample
  - 5 ml HNO_3 + 0,5 ml H_2O_2 + **20 µl HF**
  - Closed Microvawe, ramp 20 min and stay time 20 min, 170 °C
  - Dilute to 10 ml with water
  - ICP-SFMS

LIVSMEDELS VERKET
NATIONAL FOOD AGENCY

Swedish labs

- **Lab B, used by Importers**
  - Modified NMKL 186
  - 0,5 g sample
  - 8 ml HNO_3 + 2 ml H_2O_2
  - Closed Microvawe, ramp 20 min and stay time 30 min, 180 °C
  - ICP-MS

LIVSMEDELS VERKET
NATIONAL FOOD AGENCY

Different results

Sample	Importers Al mg/kg NMKL 186	NFA Al mg/kg In-house with HF
Sample A	6,48	19,6
Sample B	8,80	20,7
Sample C	6,25	22,0

≈300% higher with HF

Importers not happy

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NATIONAL FOOD AGENCY

Spiking of samples

Sample	Al mg/kg NMKL 186	Al mg/kg In-house with HF
Sample A	112%	114%
Sample B	107%	87%
Sample C	110%	96%

No problem detected

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No standard method

- Ad-hoc group lead by Dr. Peter Fecher has done some work in CEN/TC275/WG10, *Elements and their chemical species*
- *Dilution instead of use of hydrofluoric acid*
- *Not an easy task*

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Statistical Methods for Proficiency Testing

EC/IRMM Training

20-21 September, 2012

Dan Tholen, M.S.

ISO/IEC 13528 Seminar

- History of ISO 13528
- Main points of the Standard
  - Design
  - Homogeneity and Stability
  - Example Reports
- Revision in ISO TC69
  - main revisions
- Next steps

Documents for PT Statistics

- ISO/IEC 17043: 2010 *Conformity Assessment – General requirements for proficiency testing*
- ISO 13528: 2005 *Statistical Methods for use in proficiency testing by interlaboratory comparisons*
  - *Based on ISO/IEC Guide 43-1: 1997, Annex B on Statistical Methods*

Other Documents for PT Statistics

- *The International Harmonized Protocol for Proficiency Testing of Analytical Chemistry Laboratories* (IUPAC Technical Report) 2006
(http://www.iupac.org/publications/pac/2006/pdf/7801x0145.pdf)

- *IUPAC/CITAC Guide: Selection and use of proficiency testing schemes for a limited number of participants – chemical analytical laboratories (IUPAC Technical Report) 2010*
(http://iupac.org/publications/pac/pdf/2010/pdf/8205x1099.pdf)

ISO 13528

- Written by ISO TC69, SC6
- Approved work item in 1997
- Published in 2005, reaffirmed in 2009
- Now under revision, request of ILAC
- Approved as CD, in process as DIS
- To be discussed in June, 2013
- Published in 2014?

ISO 13528:2005

- Written as a Standard – many “shalls”
  - Used as guidance
- Complementary to ISO/IEC Guide 43 providing detailed guidance that is lacking
- Main objective, for statisticians, is to evaluate laboratory’s bias

  Basic Model:

  $x_i = \mu + B_i + \varepsilon$

ISO 13528:2005

- High interest / some parts are widely used
  - Of high interest in Europe
  - Followed closely in Asia
  - Followed by some medical PT (EQA)
- Goal is to describe optimal procedures, but other procedures are allowed :
  - Statistically valid, fully described to participants

ISO 13528:2005

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ISO 13528 Main Points

- Design considerations
  - Number of significant digits, replicates
- Homogeneity and Stability
- Graphical techniques

ISO 13528 Main Points

- Determining the assigned value
  - Mean of participants
  - Reference value
- Determining allowance for error
  - SD of participants
  - Determined by fitness criteria
- Performance statistics
  - z, z', En, D, D%
  - Criteria for evaluation of statistics

ISO 13528 – Examples

- APLAC, Melamine
- IMEP

ISO 13528 – Some problems

- Main objective, for statisticians, is to estimate laboratory's bias, not to evaluate performance on a single result
- Has led to requirements for PT that are different than what lab would report
  - Number of replicates
  - Number of significant digits
  - Truncated 'less than' (<) values

ISO 13528 – Some problems

- ISO Guide 43-1 no longer valid
- Applicable to quantitative data but not qualitative data.
- Some errors, incomplete descriptions
- New procedures are available
  - IUPAC Harmonized protocol, ISO Guide 35
  - More appreciation for uncertainty
  - New statistics (e.g., zeta)

ISO 13528 – Revision

- Fix problems listed
  - Correct errors, enhance descriptions
  - Considerations for qualitative data
- Retain widely applied guidelines
  - Robust procedure Algorithm A
  - Procedure for Homogeneity and Stability
  - Update to new ISO/IEC 17043
  - Add procedures for new design requirements
  - Add guidance for inspection, individuals

ISO 13528 – Revision

- Add new robust procedures
  - Simple – median, nIQR
  - Complicated – Hampel Q
- Add new performance statistics
  - Enhance use of D, D%, add P_A
  - Add zeta
- Add considerations for simplified homogeneity and stability
- Add considerations for uncertainty

ISO 13528 – Revision

- Reorder sections for usual work order
  - Design
  - Verify valid PT items
  - Review Data for expectation
  - Process data according to the design
- Move examples to informative annex
  - Add comprehensive example(s)

ISO 13528 – Revision

- New Title
  - "Interlaboratory comparison" is in the definition of PT

  "Statistical methods for proficiency testing"

- Change name of SDPA
  - It is often not a standard deviation

  "Standard Error for Proficiency Assessment"

  (other preferred?)

Three Basic Approaches for Evaluation of Performance

- Compare performance to other participants
- Compare performance against fitness for purpose criteria
- Compare performance against participant's claims for uncertainty

Three Basic Approaches for Evaluation of Performance

- Compare performance to other participants
  - Consensus mean and SD
  - z score
  - Check whether reasonable

Three Basic Approaches for Evaluation of Performance

- Compare performance against fitness for purpose criteria
  - Assigned value from reference or experts
  - SD or other criterion form external source
  - z score with reference SDPA (SEPA)
  - z' if large uncertainty of assigned value
  - D or D% and δ_E

Three Basic Approaches for Evaluation of Performance

- Compare performance against participant's claims for uncertainty
  - Assigned value from reference
  - No participant SD or SDPA
  - Zeta or En scores
  - Assumes correct evaluation of uncertainty

Enhanced use of D and D%

- $D = (x_i - X)$ $D\% = (x_i - X) / X * 100$
- Error criterion δ_E in same units as x_i or %
- δ_E is intuitive for many participants
- Common in US environmental and other
- Popular for medical in many countries
- Could transform to 'percent of allowed error' or P_A for a standardized score
- $P_A = D/\delta_E * 100$ and compare to 100%

Expansion of Uncertainty

- Uncertainty of assigned value
  - If reference value, consider homogeneity, stability, transport (similar to CRM)
  - If consensus, OK as is
- Uncertainty of AV added to z = z'
- Review participants' u_{lab}
- Use zeta or En

ISO/IEC 17043
Requirements relating to statistics

- 4.4 Design of proficiency testing scheme
  - 4.4.1 Planning
    - 4.4.1.3 The PTP shall document ...the following information..
  - p) detailed description of the statistical analysis to be used;
  - q) the origin, metrological traceability and measurement uncertainty of any assigned values;
  - r) criteria for the evaluation of performance of participants;
  - s) a description of the data, interim reports or information to be returned to participants;
- 4.4.1.4 The PTP shall be access to the necessary technical expertise and experience in …. statistics

ISO/IEC 17043

- **4.4.3 Homogeneity and stability**

4.4.3.2 The procedure for the assessment of homogeneity and stability shall be documented and conducted, where applicable, in accordance with appropriate statistical designs. Where possible, the PTP shall use a statistically random selection of a representative number of proficiency testing items from the whole batch of test material in order to assess the homogeneity of the material.

ISO/IEC 17043

- **4.4.4 Statistical design**
- 4.4.4.1 Statistical designs shall be developed to meet the objectives of the scheme, based on the nature of the data (quantitative or qualitative, including ordinal and categorical), statistical assumptions, the nature of errors, and the expected number of results

ISO/IEC 17043

- **4.4.4 Statistical design (cont'd)**

NOTE 1 Statistical design covers the process of planning, collection, analysis and reporting of the proficiency testing scheme data. Statistical designs are often based on objectives for the proficiency testing scheme, such as detection of certain types of errors with specified power or determination of assigned values with specified measurement uncertainty

NOTE 2 Data analysis methods could vary from the very simple (e.g. descriptive statistics) to complex, using statistical models with probabilistic assumptions or combinations of results for difference proficiency test items

ISO/IEC 17043

- **4.4.4 Statistical design (cont'd)**

NOTE 3 In cases where the proficiency testing scheme design is mandated by a specification given by, for example, a customer, regulatory authority or accreditation body, the statistical design and data analysis methods can be taken directly from the specification

NOTE 4 In the absence of reliable information needed to produce a statistical design, a preliminary interlaboratory comparison can be used

ISO/IEC 17043

- **4.4.4 Statistical design (cont'd)**
- 4.4.4.2 The PTP shall document the statistical design and data analysis methods to be used to identify the assigned value and evaluate participant results, and shall provide a description of the reasons for their selection and assumptions upon which they are based. The PTP shall be able to demonstrate that statistical assumptions are reasonable and that statistical analyses are carried out in accordance with prescribed procedures

ISO/IEC 17043

- **4.4.4 Statistical design (cont'd)**
- 4.4.4.3 In designing a statistical analysis, the PTP shall give careful consideration to the following:

a) The accuracy (trueness and precision) as well as the measurement uncertainty required or expected for each measurand or characteristic in the proficiency testing;
b) The minimum number of participants in the proficiency testing scheme needed to meet the objectives of the statistical design; in cases where there is an insufficient number of participants to meet these objectives or to produce statistically meaningful analysis of results, the PTP shall document, and provide to participants, details of the alternative approaches used to assess participant performance;

ISO/IEC 17043

- **4.4.4 Statistical design (cont'd)**

c) The relevance of significant figures to the reported results, including the number of decimal places;
d) The number of proficiency test items to be tested or measured and the number of repeat tests, calibrations or measurements to be conducted on each proficiency test item or for each determination;
e) The procedures used to establish the standard deviation for proficiency assessment or other evaluation criteria;
f) Procedures to be used to identify or handle outliers, or both;
g) Where relevant, the procedures for the evaluation of values excluded from statistical analysis; and
h) Where appropriate, the objectives to be met for the design and the frequency of proficiency testing rounds.

ISO/IEC 17043

- **4.4.5 Assigned values**

4.4.5.1 The proficiency testing provider shall document the procedure for determining the assigned values for the measurands or characteristics in a particular proficiency testing scheme. This procedure shall take into account the metrological traceability and measurement uncertainty required to demonstrate that the proficiency testing scheme is fit for its purpose.

4.4.5.4 When a consensus value is used as the assigned value, the PTP shall document the reason for that selection and shall estimate the uncertainty of the assigned value as described in the plan for the proficiency testing scheme

ISO/IEC 17043

- **4.7 Data analysis and evaluation of proficiency testing scheme results**

4.7.1 Data analysis and records

4.7.1.2 Results received from participants shall be recorded and analysed by appropriate methods. Procedures shall be established and implemented to check the validity of data entry, data transfer, statistical analysis, and reporting.

4.7.1.3 Data analysis shall generate summary statistics and performance statistics, and associated information consistent with the statistical design of the proficiency testing scheme.

ISO/IEC 17043

- **4.7 Data analysis and evaluation of proficiency testing scheme results (cont'd)**

4.7.1 Data analysis and records

4.7.1.4 The influence of outliers on summary statistics shall be minimized by the use of robust statistical methods or appropriate tests to detect statistical outliers.

4.7.1.5 The PTP shall have documented criteria and procedures for dealing with test results that may be inappropriate for statistical evaluation, e.g. miscalculations, transpositions and other gross errors.

ISO/IEC 17043

- **4.7 Data analysis and evaluation of proficiency testing scheme results (cont'd)**

4.7.2 Evaluation of performance

4.7.2.1 The PTP shall use valid methods of evaluation which meet the purpose of the proficiency testing scheme. The methods shall be documented and include a description of the basis for the evaluation….

ISO/IEC 17043

- **4.8 Reports**

4.8.2 Report shall include the following, unless it is not applicable or the PTP has valid reasons for not doing so:
….
k) statistical data and summaries, including assigned values and range of acceptable results and graphical displays:
….
n) procedures used to establish the standard deviation for proficiency assessment, or other criteria for evaluation;

ISO/IEC 17043

- **4.8 Reports (cont'd)**

….

o) assigned values and summary statistics for test methods/procedures used by each group of participants (if different methods are used by different groups of participants);

….

r) procedures used to statistically analyse the data;

….

ISO/IEC 17043 Annex B (informative)
Statistical methods for proficiency testing

- The statistical methods used to analyse the results need to be appropriate for each situation, and so are too varied to be specified in this International Standard.
- ISO 13528 describes preferred specific methods for each of the situations discussed below, but also states that other methods may be used as long as they are statistically valid and are fully described to participants.

ISO/IEC 17043 Annex B (informative)
Statistical methods for proficiency testing

- Some of the methods in ISO 13528, especially for homogeneity and stability testing, are modified slightly in the IUPAC Technical Report "*The International Harmonized Protocol for the proficiency testing of analytical chemistry laboratories*"
- These documents also present guidance on design and visual data analysis.
- Other references may be consulted for specific types of proficiency testing schemes, e.g. measurement comparison schemes for calibration

ISO/IEC 17043 Annex B (informative)
Statistical methods for proficiency testing

- Fundamental steps common to nearly all proficiency testing schemes:
  - Determination of the assigned value
  - Calculation of performance statistics
  - Evaluation of performance
  - Preliminary determination of proficiency test item homogeneity and stability

ISO/IEC 17043 Annex B (informative)
Statistical methods for proficiency testing

Determination of the assigned value and its uncertainty

Procedures available:

- *Known values* – formulation (e.g. manufacture or dilution)
- *Certified reference values* – by definitive methods
- *Reference values* - determined by comparison alongside a reference material or standard traceable to a national or international standard
- *Consensus value from expert participants* (e.g. reference labs)
- *Consensus values from participants*

- All these are for quantitative data

ISO/IEC 17043 Annex B (informative)
Statistical methods for proficiency testing

Determination of the assigned value and its uncertainty (cont'd)

- Other considerations:
  - If consensus, control outliers
  - If consensus, check trueness of process
  - Criteria for acceptability on the basis of uncertainty of the assigned value (for all a.v., especially consensus)

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency testing

Determination of the assigned value and its uncertainty (cont'd)

- Outliers are statistically treated as described below.
  - Obvious blunders, such as those with incorrect unit, decimal errors, and results for a different proficiency test item should be removed from the data set and treated separately. These results should not be subject to outlier tests or robust statistical methods

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency testing

B2 Determination of the assigned value and its uncertainty (cont'd)

- When participants' results are used to determine assigned values, statistical methods should be in place to minimize the influence of outliers. This can be accomplished with robust statistical methods or by removing outliers prior to calculation. In larger or routine proficiency testing schemes, it may be possible to have automated outlier screens, if justified by objective evidence of effectiveness

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency testing

B2 Determination of the assigned value and its uncertainty (cont'd)

- If results are removed as outliers, they should be removed only for calculation of summary statistics. These results should still be evaluated within the proficiency testing scheme and be given the appropriate performance evaluation

  NOTE ISO 13528 describes a specific robust method for determination of the consensus mean and standard deviation, with the need for outlier removal.

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency testing

Determination of the assigned value for qualitative data

- Statistical methods for determining the assigned values for qualitative data or semi-qualitative values are not discussed in ISO 13528
- These assigned values need to be determined by expert judgment or manufacture

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency testing

Determination of the assigned value for qualitative data

- Consensus value, as defined by agreement of a predetermined majority percentage of responses (e.g. 80% or more)
- Percentage used should be determined based on objectives for the PT scheme and the level of competence and experience of the participants
- May use median or mode for ordinal data, not mean

ISO/IEC 17043 Annex B (informative) Statistical methods for proficiency

- No such thing as standard deviation for ordinal data
- IT IS NOT APPROPRIATE to calculate the mean or SD of semi-quantitative values.

Qualitative Data (ISO/IEC 17043)

- Categorical or Nominal (e.g., present/absent):
  - Usually assigned value is by expert judgment
  - Can use mode as assigned value
- Ordinal (semi-quantitative)
  - Preferred to use expert judgment as assigned value
  - Can use median or mode
  - DO NOT USE THE MEAN (undefined distribution)

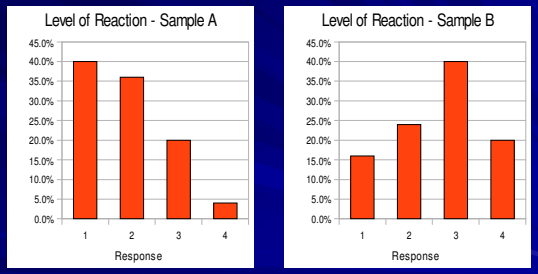
Example – Semi-Quantitative (ordinal)

- Measurand: Level of reaction, by category:
  - 1 = no reaction, normal
  - 2 = mild reaction
  - 3 = moderate reaction
  - 4 = severe reaction
- 2 PT samples, A and B
- 50 participants

Example – Semi-Quantitative

- Sample A:
  - 1 = 20 results (40%)
  - 2 = 18 results (36%)
  - 3 = 10 results (20%)
  - 4 = 2 results (4%)
- Sample B
  - 1= 8 results (16%)
  - 2 = 12 results (24%)
  - 3 = 20 results (40%)
  - 4 = 10 results (20%)

Responses for Samples A and B



13528 - Robust Analysis

- Robust statistical method
  Statistical method insensitive to small departures from underlying assumptions surrounding an underlying probabilistic model
- A way of summarizing results when we suspect that they include a small proportion of outliers

13528 - Robust Analysis

- Mean of all results is not robust because it can be affected a single very large/small outlying datum
- Breakdown point – proportion of incorrect observations the estimator can handle before giving a biased arbitrary mean
- Range from 0 to 0.5

13528 - Robust Analysis

- Calculated by downweighting the data points that are distant from the mean and then compensating for the downweighting
- Examples are median and Huber robust mean (Algorithm A)
- Algorithm A makes more use of the information in the data than the median does and consequently has a smaller standard error
- Median is more robust when the frequency distribution is strongly skewed

13528 - Robust Analysis

- Robust mean is preferred when the distribution is close to symmetric
- Huber's method progressively transform the original data by a process call winsorisation
- The transformation of statistics by limiting extreme values in the statistical data to reduce the effect of possibly spurious outliers
- Data are not discarded but replaced by certain statistical minimum and maximum

13528 - Robust Analysis

- Assume that we have initial estimate of median (x^*) and robust standard deviation ($s^* = 1.483 \times MAD$)
- If a value x_i falls above ($x^* + 1.5\ s^*$) , then we change it to ($x^* + 1.5\ s^*$)
- If a value x_i falls below ($x^* - 1.5\ s^*$) , then we change it to ($x^* - 1.5\ s^*$)
- Otherwise [*i.e. for all data lying between ($x^* + 1.5\ s^*$) and ($x^* - 1.5\ s^*$)*] we do not change the data
- Then calculate improved estimate mean of the transformed data and a std dev using a formula [1.134 X stddev (transformed data)]

13528 - Robust Analysis

- Algorithm A for mean and SD
  Starts with $x^* = \text{median}$
  $s^* = 1.483 \times \text{median}|x_i - x^*|$

  Limit data at $x^* + 1.5s^*$ and $x^* - 1.5s^*$
  Extreme values revised to $1.5s^*$

13528 - Robust Analysis

- Calculate new: $x^* = (\Sigma x_i)/p$
  $s^* = 1.134\sqrt{\Sigma(x_i^* - x^*)^2/(p-1)}$

Revise data again, at $1.5s^*$
Recalculate new x^* and s^*
Repeat until convergence

13528 - Robust Analysis

Convergence assumed when there is no change from one iteration to the next in the third significant figure of the robust standard deviation and of the equivalent figure in the robust average

13528 - Robust Analysis

- Robust methods assume that the underlying distribution roughly normally (and therefore unimodal and symmetric) but contaminated with outliers and heavy tails
- Give misleading results if they are applied to data sets that are markedly skewed or multimodal, or if a large proportion of the data are identical in value

Removal of outliers

- Acceptable but not preferred by 13528
- Obvious blunders, such as those with incorrect units, decimal point errors, results for a different proficiency test item removed and not subject to outlier tests
- If results are removed as outliers, they should be removed only for calculation of summary statistics only but should be evaluated and given the appropriate evaluation

Removal of outliers

- Grubbs' test
- Use to determine whether the largest or smallest datum in a set is an outlier

For largest value:

$G_h=(x_h-x)/s$

For smallest value:

$G_l=(x-x_l)/s$

These values are compared with critical ones of Grubb's test

- Grubbs is valid for specific number of outliers
- There are other outlier tests

Limiting the uncertainty of the Assigned Value (X): 13528 Section 4.2

- Establish limits for uncertainty of AV

$u(X) < 0.3\sigma_P$

When using fixed limits (E)…

$u(X) < 0.3(E/3)$

$u(X) < E/10$

Limiting the uncertainty of the Assigned Value (X): 13528 Section 4.2

- If this cannot be met then
  - Look for a better way to determine AV
  - Incorporate uncertainty in score
    - z'
    - E_n
    - zeta
  - Advise participants of large uncertainty

Determining Performance Scores

- Three general approaches for scoring PT
  1. Relative to pre-determined criteria
     - Fitness for purpose
     - Expert expectation
  2. Relative to other participants performance
     - Z score based on participant results
  3. Relative to participants' own criteria
     - Uncertainty based approaches

Pre-determined Criteria

- Two general approaches
  1. Z score with σ_p (SDPA) prior to PT round

  1. D or D%
     - Direct comparison with criterion

Relative to Other Participants

- Traditional z score or z' score with assigned value and σ_p determined from participant results

Relative to Participant's Uncertainty

- Scores that evaluate whether PT result is close to the assigned value within the combined uncertainty of the result and the assigned value
  - E_n
  - Zeta

SD for Proficiency Assessment

- Standard deviation for proficiency assessment - σ_P (also called SDPA, in Europe)
- Measure of dispersion used in the evaluation of results of proficiency testing, based on the available information

NOTE 1 The SDPA applies only to rational and differential scale results

NOTE 2 Not all proficiency testing schemes evaluate proficiency based on the dispersion of results

SD for Proficiency Assessment

- Discussed in detail in section 6 of ISO 13528
- SD as used in z scores
- 5 approaches to get σ_P (for z scores)
  - By prescription
  - By perception
  - From a general model (e.g. Horwitz)
  - By a precision experiment (ISO 5725-2)
  - From participant data (robust SD)
- Should be chosen as fitness for purpose, under a common model for all analytes

SD for proficiency assessment

- By prescription
  - set at a value required for a specific task of data interpretation, or derived from legislation requirement
  - Advantage: relates directly to "fitness for purpose" statement

SD for proficiency assessment

- Can also be thought of as 1/3 of evaluation interval (fitness for purpose limit)
  (when z>3 is action signal)

For example if prescribed fixed interval is E = ±20%...

Then E = 3 σ_P

σ_P = E/3 = 20%/3 = 6.7%

SD for proficiency assessment

- By perception
  - set at a value corresponds to the level of performance that coordinator wishes the labs to be able to achieve
  - σ_P equivalent to a "fitness for purpose" statement
  - may not be realistic in relation to reproducibility of the measurement method

σ_p reasonableness

- Reproducibility:
  $\sigma_R = \sqrt{(\sigma_L^2 + \sigma_r^2)}$
  With:
  σ_R = Reproducibility SD
  σ_L = Between Laboratory SD
  σ_r = Repeatability SD

Reproducibility is generally considered to be a reasonable expectation for competent laboratories
Sometimes an expert technical committee will specify a σ_P that is different than σ_R

SD for proficiency assessment

- General Model – Horwitz curve (more commonly known as the "Horwitz Trumpet")

$$\sigma_P = 0.02c^{0.8495}$$

where c is the concentration of the chemical species expressed in mass fraction

SD for proficiency assessment

- General Model – Thompson's modification of Horwitz curve

$\sigma_P = 0.22\ c$ if $c < 1.2X10^{-7}$
$\sigma_P = 0.02c^{0.8495}$ if $1.2X10^{-7} \le c \le 0.138$
$\sigma_P = 0.01c^{0.5}$ if $c > 0.138$

where c is the concentration of the chemical species expressed in mass fraction

SDPA by General Model

- General Model (cont'd)
  - Disadvantage: true reproducibility may differ substantially form the value predicted by model

SDPA by Horwitz Model

- Need modification at concentration lower than about 10 ppb
- Based on a study of interlaboratory collaborative trials. Dr William Horwitz analysed the data from thousands of analytical studies (most food analysis)
- Relationship holds regardless of the nature of analyte and the test material, or the physical principle underlying the measurement method

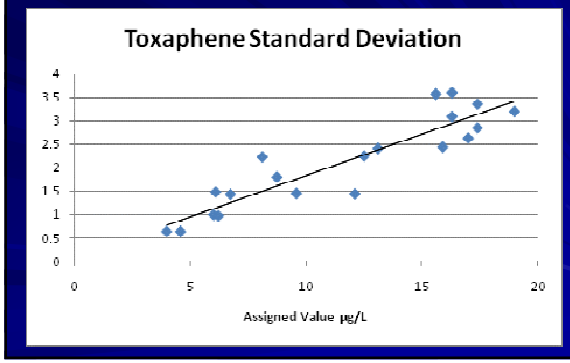
SDPA by Horwitz Model

- Not yet any widely accepted theoretical principle explaining this relationship
- Still an empirical relationship
- Cautions – not suggested for use in applications where high accuracy is required

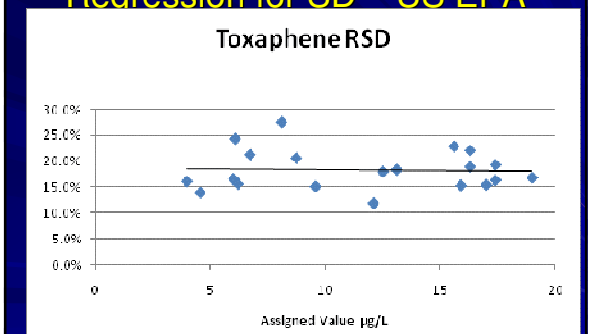
SDPA from a Model based on Experience

- If a PTP has experience with PT over time, then the robust SDs can be fit with a linear model to estimate the average SD at each level
- This average can be used to determine an SDPA that is not subject to variability across studies.

Regression for SD – US EPA



Regression for SD – US EPA



SD for proficiency assessment

- By precision experiment
  e.g ISO 5725-2
- Measurement method is a standard method and σ_R and σ_r are known
- Calculate between-lab SD using

$$\sigma_L = \sqrt{(\sigma_R^2 - \sigma_r^2)}$$

Then calculate σ_p as

$$\sigma_p = \sqrt{(\sigma_L^2 + (\sigma_r^2/n)}$$

SD for proficiency assessment

- From data obtained in a round of proficiency scheme, i.e. participant data
- Calculated by robust standard deviation of results reported by all participants using Algorithm A
- Other sound statistical methods may be used
- Disadvantage – σ_p may vary between rounds making it difficult to detect trend over several rounds

Median / nIQR Robust procedure

- Calculate Quartiles Q1, median, Q3
  IQR = Q3-Q1
  Median is an estimate of mean
  Normalized IQR is an estimate of SD
  nIQR = 0.7413 x IQR

  Note, Median Absolute Deviation (MAD) is preferred to nIQR as robust SD

Median / MAD Robust procedure

Median Absolute Deviation (MAD) is preferred to nIQR (statistically) as robust SD, but is very similar

MAD = median$|x_i - X|$

$s^* = 1.483 \cdot (MAD)$

MAD is initial s* in Algorithm A

SD for proficiency assessment

- $\sigma_{rob} > 1.2\ \sigma_p$ indicate labs are having difficulties in achieving the required reproducibility or that there are two or more populations (IUPAC Protocol)

Calculating performance statistics

- Measure deviation of a participant's result from the assigned value in a manner that allows comparison
- Quantitative results
  - D and D%
  - Z, z'
  - E_n, Zeta

Calculate Performance Statistic

Estimates of Absolute Performance:

Difference: D=(x-X)

Percentage Difference: D%=100%(x-X)/X

D and D% can be evaluated with Fixed Limits

NOTE absolute value D should NOT be used because they conceal the sign of bias

| |

Estimates of Relative Performance

– rank or percentage rank (not recommended)

– z score (recommended) $z=(x-X)/\sigma_P$

– z'

Calculate Performance Statistic

Estimates of Performance relativeto internal performance declarations:

- En
- Zeta

| |

Determine Performance Interval

- Fixed Limits (or “Fitness for Purpose”)
- Can come from methods for SD
- Not widely used
- Preferred for interpretation
  - Fixed percentage across range
  - Fixed value across range
  - Mixed or segmented.

Performance Scores

- *z*-scores

$$z = (x - X)/\sigma_P$$

- Most widely used
- Can be determined in advance σ_P that is fit for purpose and is broadly applicable to the relevant field
- Limitation: u_x and u_X not taken into account

Scores that use uncertainty

- E_n numbers (Error, normalized)
- consider expanded uncertainty of participant result and assigned value
  - – Requires consistent determination of uncertainty by all laboratories
- E_n in common use in calibration

$$E_n = (x\text{-}X)/\sqrt{(U^2_{lab}+U^2_{ref})}$$

NOTE: $U_{lab} \equiv U_x$

Scores that use uncertainty

- Also assesses the lab in choosing coverage factor k
- Support CMC claims
- When the expanded uncertainties are calculated using a coverage factor of 2.0, a critical value of 1.0 for an En number is similar to the critical value of 2.0 with z-score
- Limitation: U_{lab} must be estimated correctly

Scores that use uncertainty

- z’-scores
- uses standard uncertainty of assigned value only
  - – Useful when too much uncertainty in assigned value.
  - – Same as z when small uncertainty

$$z' = (x\text{-}X)/\sqrt{(\sigma_P{}^2+u^2_X)}$$

Scores that use uncertainty

Ratio between z-scores and z'-score

$= \sigma_P / \sqrt{(\sigma_P^2 + u^2_X)}$

z-scores always equals to or larger than z'-scores

If the uncertainty of assigned value u_X meets ISO 13528 requirement of $u_X < 0.3\sigma_P$, then this ratio should fall in the range of

$$\mathbf{0.96} \leq \sigma_P / \sqrt{(\sigma_P^2 + u^2_X)} \leq 1.00$$

and z'-scores almost identical to z-scores

Scores that use uncertainty

- Factors to consider for choosing z-scores or z'-scores
  - No benefit if $u_X < 0.3\sigma_P$
  - Use z'-scores if the above not met
  - Use z'-score if the consequences to labs are severe
- Limitation: ISO 13528 cautions that this is only valid when the participants' results are not used to determine the assigned value, because of correlation between results and assigned value.
  - Some PTPs use it anyway
  - Will be discussed in revise ISO 13528

Scores that use uncertainty

– Zeta-scores (ζ)

zeta scores (like E_n, but with std. uncertainty)

$$\zeta = (x-X)/\sqrt{(u^2_x + u^2_X)}$$

u^2_x is the lab's own estimate of its result x and

u^2_X is the standard uncertainty of the assigned value X

Scores that use uncertainty

- Zeta-scores (ζ)
  - Limitations:
    - May be used when participants' result not used to calculate the assigned value, otherwise not valid due to correlation
      - Some PT providers use Zeta anyway, will be discussed in revised ISO 13528
    - Should be used only when there is an effective system for verifying lab's own estimates of u_x (valid estimates of u_x)

Scores that use uncertainty

- Zeta-scores (ζ)
  - If such system absent, ζ-score shall be used only in conjunction with z-score as follows:
    - A lab obtains z-scores repeatedly exceed 3.0
    - Lab examine its procedure to identify steps with largest uncertainties
    - Effort put to improve these identified steps
    - ζ-score repeated exceed 3.0, implies their uncertainty budget is underestimated

Scores that use uncertainty

- Zeta-scores (ζ)
  - Can be useful in combination with z score
  - Z score grades relative to other participants when X and/or σ_P determined by consensus
  - Z score grades relative to fitness for purpose when σ_P is determined as fitness for purpose
  - Zeta score grades relative to individual laboratory capabilities

Scores that use uncertainty

- E_z score (might be removed from revised ISO 13528)

$E_{z-} = x - (X - U^2_X)/U_x$

$E_{z+} = x-(X + U^2_X)/U_x$

U_X = expanded uncertainty of assigned value
U_x = expanded uncertainty of lab's result

Scores that use uncertainty

– E_n score ("Error, normalized")

$E_n = (x-X)/\sqrt{(U^2_{lab}+U^2_{ref})}$

– z' scores (like z, includes u_x)

$z' = (x-X)/\sqrt{(\sigma_p{}^2+u^2_X)}$

– zeta scores (like E_n, but with std. uncertainty)

$\zeta = (x-X)/\sqrt{(u^2_x+u^2_X)}$

Homogeneity and Stability

Demonstration of homogeneity and stability in ISO/IEC 17043

- Ensure sufficient homogeneity so as to not impact evaluation of performance
- Different needs for determining H&S in PT and in for Reference Materials (ISO Guides 34 and 35)
  - PT (and RM) needs to ensure sufficient
  - CRM needs to estimate SD between samples, and instability as part of uncertainty of assigned value

Homogeneity – ISO 13528

- Homogeneity
  - Precision of method: $(\sigma_{an} / \sigma_P) < 0.5$
  - 10 or more samples, 2 replicates
  - SD_S for samples (ANOVA or direct calculation)
  - $SD_S < 0.3\ \sigma_P$
  - No F test
- Can use experience to reduce testing

When evidence and theory prove homogeneous

Homogeneity – IUPAC (2006)

- Similar to ISO 13528, larger criterion for acceptance, more complex statistics.
- 10 or more samples, in duplicate
- Sufficient repeatability: $\sigma_{an} < 0.5\sigma_p$
- Cochran test for duplicates
- Visual check for anomalies
  - Non-random differences between replicates
  - Time trend across manufacture

Homogeneity – IUPAC (2006)

- Calculate variances
  - S^2_{an} (between replicates)
  - S^2_{sam} (between samples)
  - $\sigma^2_{all} = (0.3\sigma_p)^2$
- Calculate acceptance criterion
  - Take F_1 and F_2 from Tables
  - $c = F_1\sigma^2_{all} + F_2 s^2_{an}$
  - If $S^2_{sam} < c$ then acceptable homogeneity
- Since $F_1>0$ and $s^2_{an}>0$ and σ^2_{all} = 13528 criterion, this is always an easier criterion

Homogeneity - traditional

- F test (allowed, not recommended)

  $F = (SD_S^2/s_r^2)$

  S_r = repeatability SD_S= between samples

  $F_{crit} = F_{(.05,k-1,\ s(n-1))}$ k=# samples n=# replicates

  - High S_r → insensitive test (large SD_S passes)
  - Low S_r → too sensitive test (small SD_S fails)

Stability – ISO 13528

- Stability
  - Analysis on or after closing date
  - (2-)3 samples, (1-)2 replicates, depending on experience
  - Calculate overall mean
  - [Mean(H) – Mean(S)] < 0.3 σ_P
  - No statistical *t* test
    - High S_r → insensitive test (big difference passes)
    - Low S_r → too sensitive test (small difference fails)

Stability - practical

- Can use experience and technical knowledge (backed by data)
  - Same measurand, same manufacture process, same matrix
  - For calibration artefacts, homogeneity and stability are usually the same thing

End

Work package 3

DTU Food
National Food Institute

WP leader
Jens J. Sloth

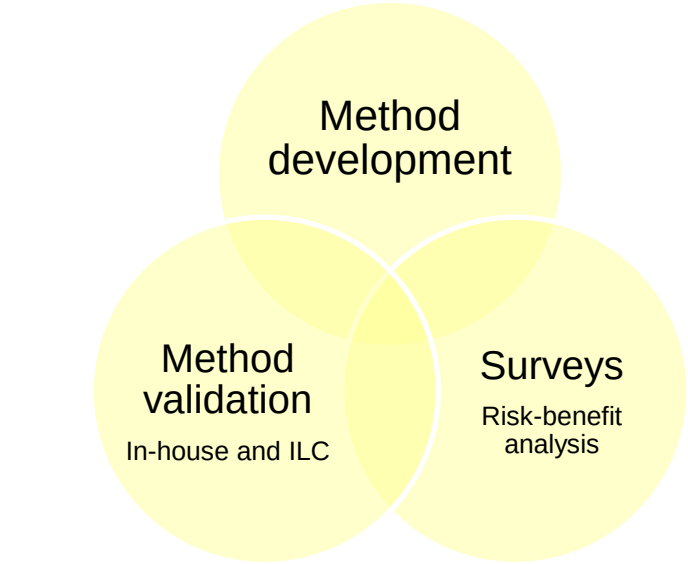
Heavy metals

CONffIDENCE
Stakeholder workshop
Brussels 20. September 2012




Agenda

- **The CONffIDENCE project – general information**
- **WP3 on ”heavy metals” – in focus**
- **Inorganic arsenic**
  - SPE HG-AAS method
  - seafood samples
  - rice samples
- **Methylmercury**
  - HPLC-ICPMS method
  - seafood samples
  - feed samples



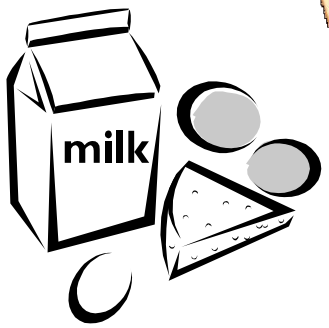
CONffIDENCE in a nutshell

CONtaminants in **F**ood and **F**eed –
Inexpensive **DE**tectio**N** for **C**ontrol of **E**xposure

- Collaborative Project: FP7 (European Commission)
- Duration: May 2008 – Dec 2012
- 16 partners from 10 countries, representing universities, research institutes, industry and SMEs
- Budget: 7.5 Mio €
- Coordinator: RIKILT - Institute of Food Safety, part of Wageningen UR (NL)
- WP3 leader: DTU Food

The commodities

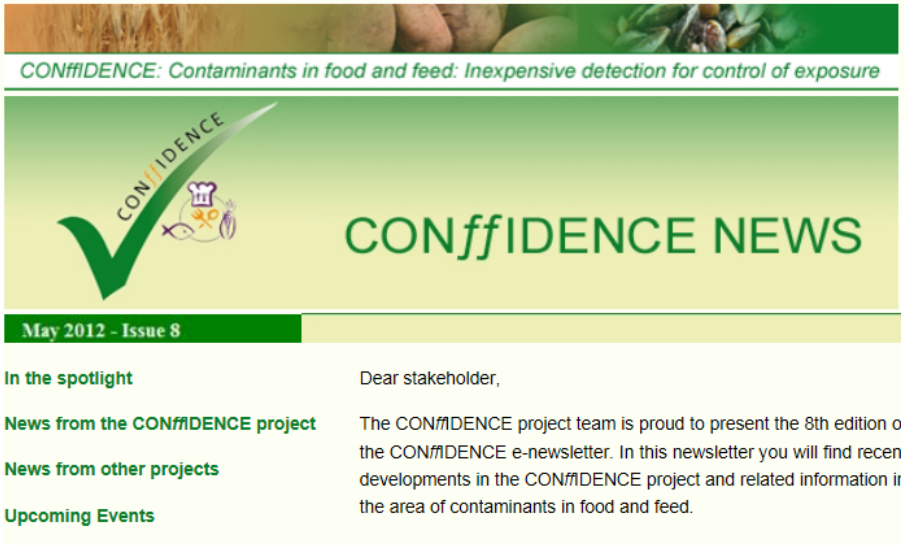
- **Food & Feed**
- Fish/shellfish and fish feed
- Cereals and cereal-based feed
- Potatoes/vegetables
- Honey
- Eggs
- Meat
- Dairy products



The target contaminants

- POPs: - dioxin-like PCBs + metabolites
  - brominated flame retardants
  - polycyclic aromatic hydrocarbons (PAH)
- Perfluorinated compounds (PFCs)
- Pesticides: paraquat/diquat, dithiocabamates
- Veterinary drugs: - antibiotics, e.g. tetracyclines
  - coccidiostats, e.g. ionophores
- **Heavy metals speciation: -inorganic arsenic**
  -methylmercury
- Biotoxins: - alkaloids
  - marine biotoxins
  - mycotoxins

www.conffidence.eu



Newsletter –
2 times/year

WP3 overall objectives

Objectives

Development of simplified methodologies for the determination of

1) **inorganic arsenic (iAs)** in seafood
2) **methylmercury (MeHg)** in marine based food and feed.

2 parallel approaches were followed

1) **cytosensor** approach using luminescent bacterial cell biosensor

2) **solid phase extraction** approach followed by AAS (SPE-AAS)

WP3 - relevance

- Current situation in EU legislation:

Foodstuffs
MLs for Pb, Cd, Hg and Sn
EU directive 2006/1881/EC (and amendments)

Animal feedingstuffs
MLs for As, Pb, Cd and Hg
EU directive 2002/32/EC (and amendments)

Only maximum levels for total concentration of the metals

Arsenic

- inorganic As (iAs) is the toxic form of As
- Lack of specific data on iAs (*EFSA, 2009 and JECFA, 2010*)
- Lack of validated, standardised methods *(EFSA, JECFA)*

Mercury

- Methylmercury is considered more toxic than inorganic Hg (iHg)

Seafood/marine feed

- Seafood is the predominant source of As and Hg in the European diet
- Focus on marine feed and food sample types

EFSA (2009) and JECFA (2010) opinions on arsenic in food

- Old PTWI value (WHO, 1988) was withdrawn

- **NEW!** $BMDL_{1.0}$ = 0.3 – 8 µg/kg bw per day for inorganic arsenic
- => EU dietary exposures within this range
- => Risk to some consumers cannot be excluded

- **NEW!** $BMDL_{0.5}$ = **3 µg/kg bw per day** for inorganic arsenic
  => 0.5% increased incidence of lung cancer for 12 y exposure

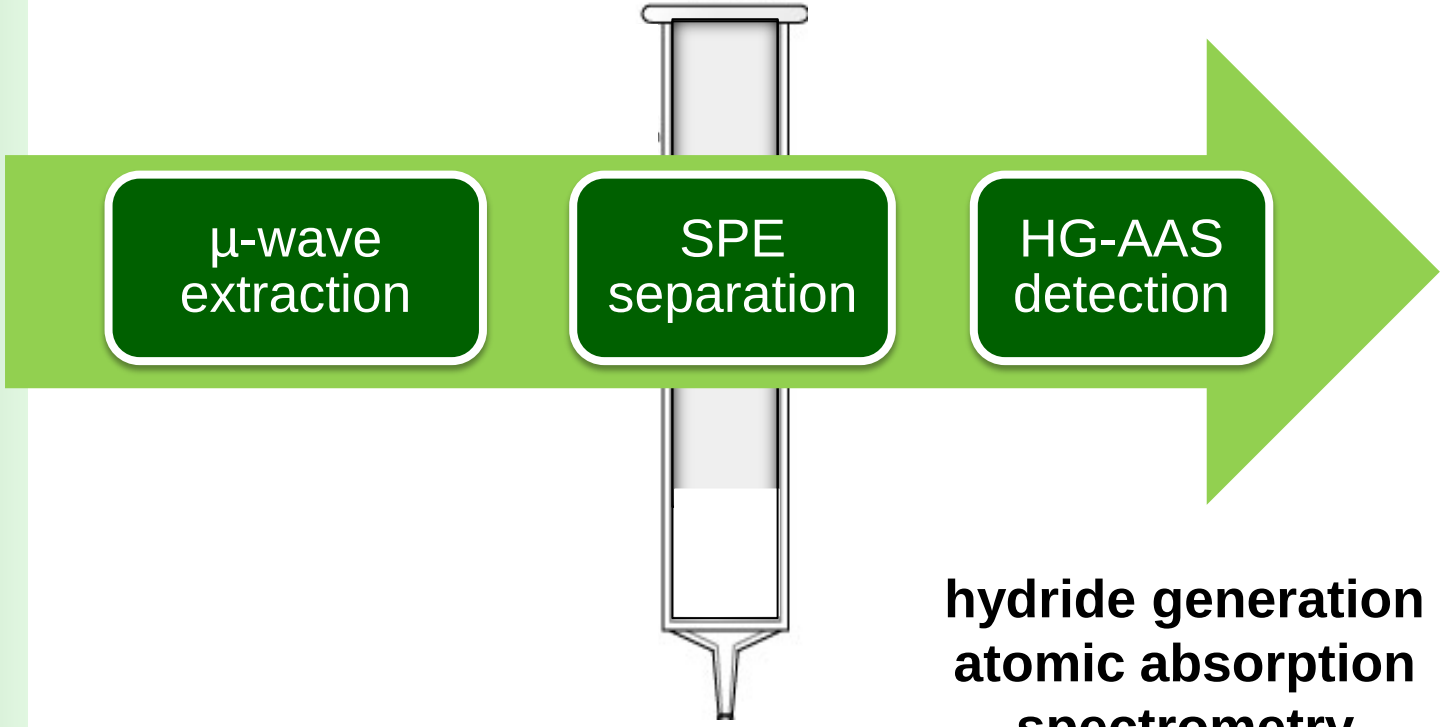
- *"…there is a need to produce speciation data for different food commodities to support dietary exposure assessment…"*

- *"…more accurate information on the inorganic arsenic content of foods is needed to improve assessments of dietary exposures to inorganic arsenic"*

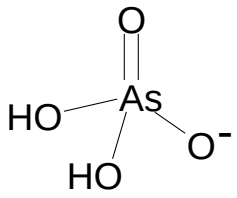
- *"…need for validated methods for selective determination of inorganic arsenic in food matrices"*

Arsenic speciation analysis

speciation alternative: SPE, HG-AAS



hydride generation atomic absorption spectrometry



inorganic arsenic

µ-wave extraction - oxidation of As(III) to As(V)

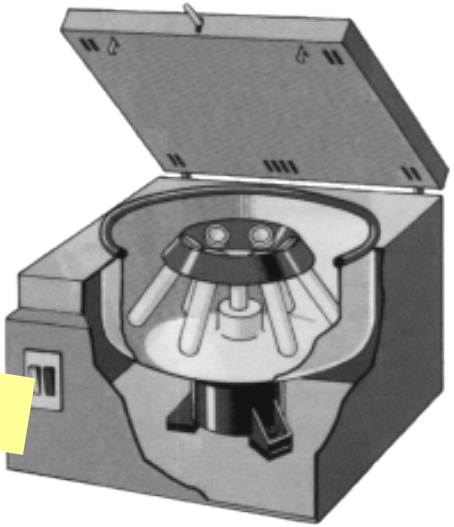
0.2 g sample
+ 10 mL extractant
(0.06 M HCl, 3% H_2O_2)

25 minutes at 90°C

Centrifugation
10 min 2100 x *g*

Glas vessel

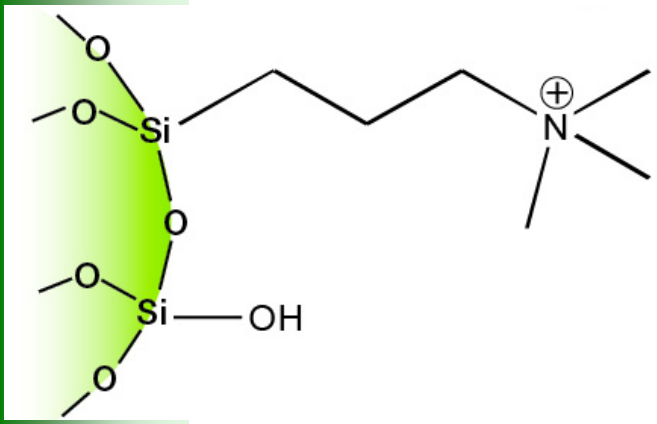
µ-wave oven

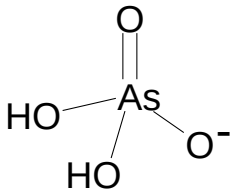


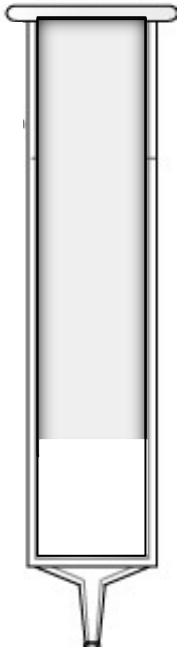
centrifuge

Quantitative conversion: As(III) -> As(V)

SPE protocol - separation of As species







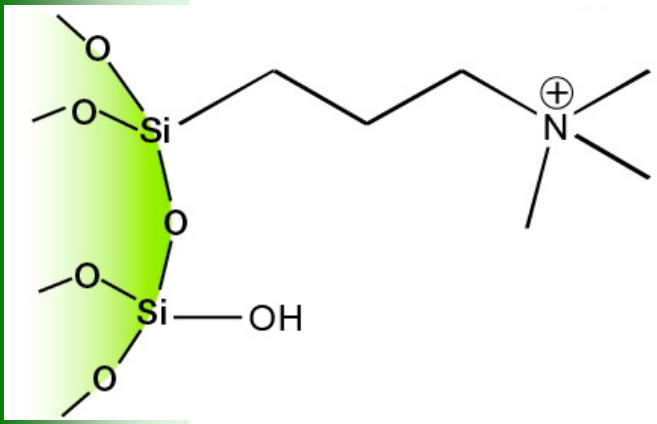
Strong anion exchange SPE column
silica based
Strata SAX
500 mg/6 mL, Phenomenex

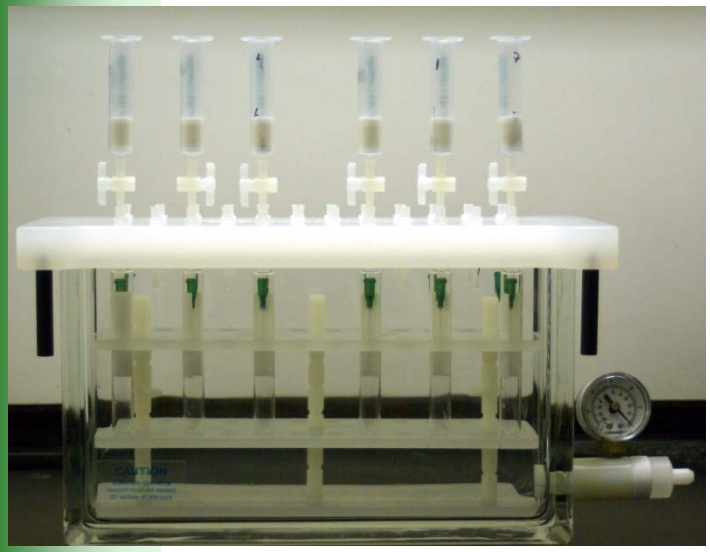
The **charge** of the arsenic species depends on pH

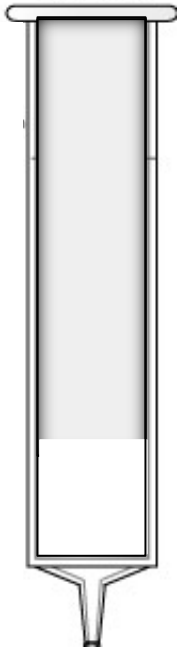
@ pH = 6 **iAs(V)** is **negatively charged**

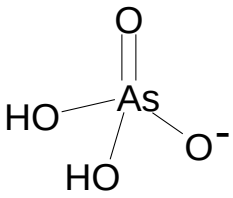
Sequential elution
Separation of inorganic As from organo As species by SPE

SPE protocol - Separation of As species









Condition
100 % MeOH

Equilibrate
Buffer: 20mM $(NH4)_2CO_3$, 0.03 M HCl and 1.5% H_2O_2

Load
Buffered sample: pH 5.0-7.5

Wash 0.5 M CH_3COOH

Elute 0.5 M HCl

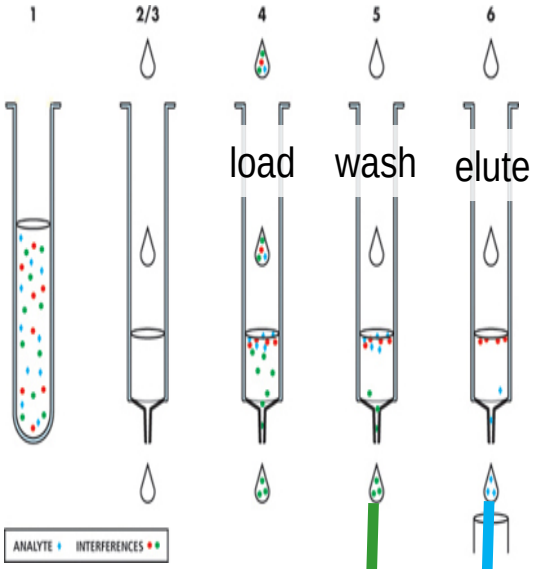
SPE-HG-AAS – a novel speciation alternative…

μ-wave extraction

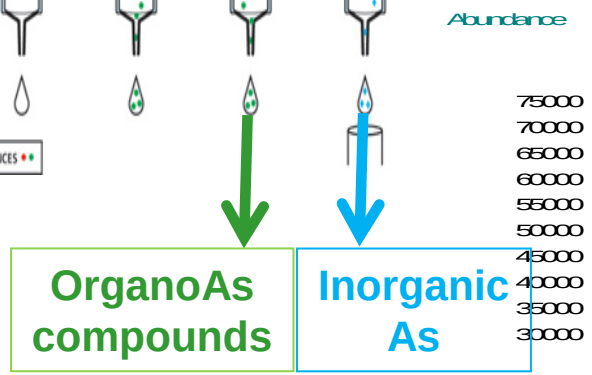
Separation by SPE

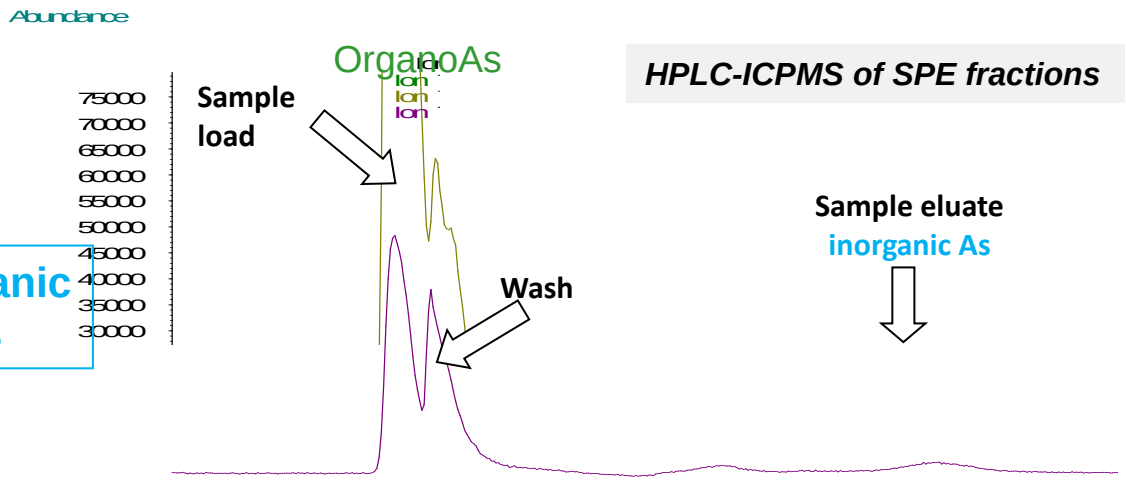
Detection by HG-AAS

Inexpensive detection system



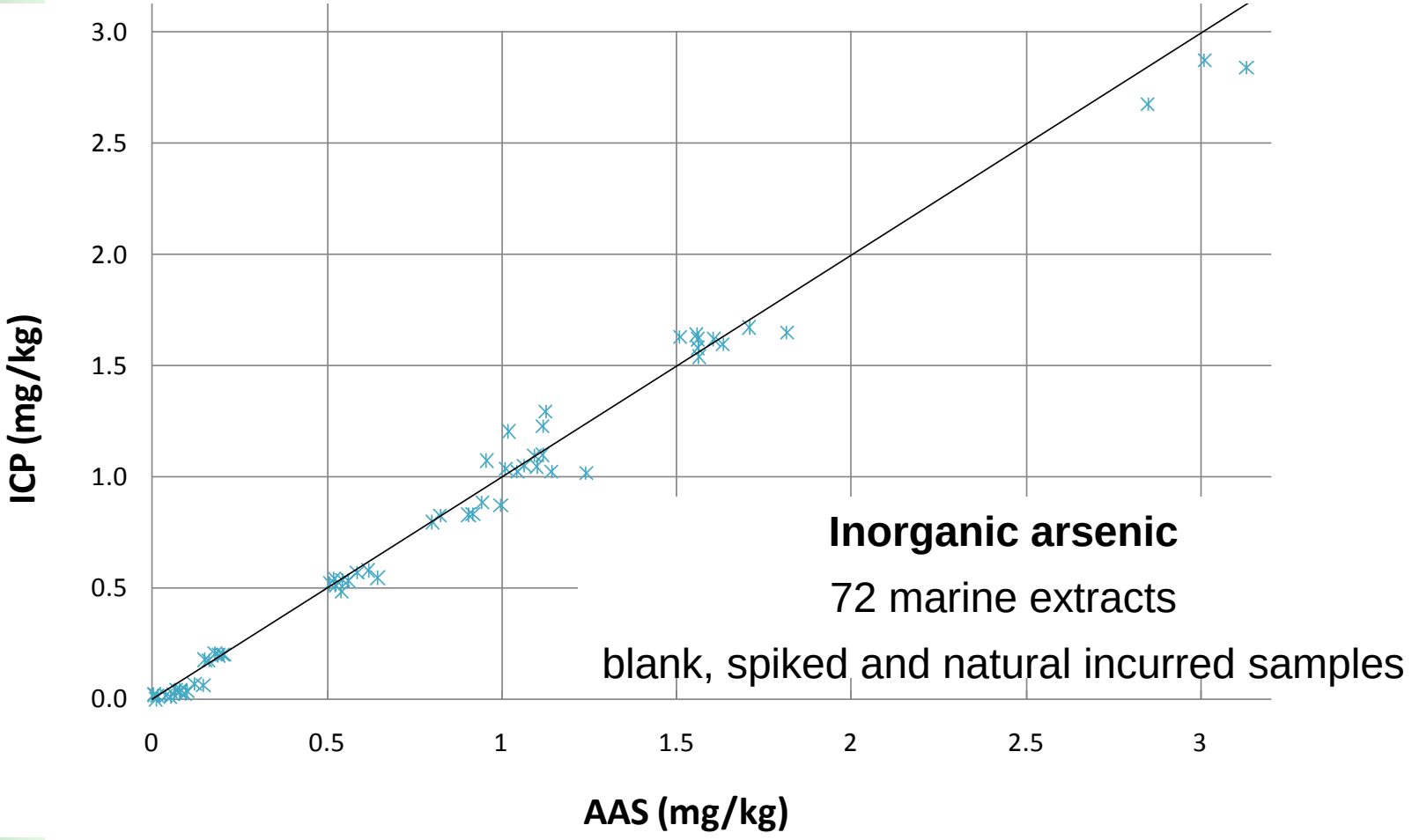
Sequential elution for selective off-line separation of inorg As from organo As species by SPE





HPLC-ICPMS of SPE fractions

Inorganic arsenic: SPE-HG-AAS versus HPLC-ICP-MS



The detection methods were not significantly different (*t* Test, 95% confidence)

In-house validation – iAs by SPE-HGAAS

Setup

- Spiked samples → Trout, oyster
- Natural incurred samples → TORT-2, DORM-3
- Analysed in triplicates on 3 different days
- 2 technicians

Results overview

- 0.08 mg/kg limit of detection (LOD)
- 3-8% repeatability
- 5-13% reproducibility
- 90-104% recovery

	Spike low	Spike medium	Spike high	TORT-2	DORM-3
iAs level (mg/kg)	0.5	1	1.5	0.9*	0.2*
Observations (N)	9	9	9	6	6
Mean recovery (%)	101	103	104	100	90
Repeatability RSD_r (%)	4	8	5	3	7
Reproducibility RSD_{IR} (%)	5	9	6	9	13
Horwitz Rel. Std. (%)	18	16	15	16	20

**Reference value determined by HPLC-ICP-MS*

Rasmussen et al, ABC 2012

Collaborative trial – marine samples

Sample	Description	~conc level (mg/kg)
WP3-2	IMEP32-4 fish meal spiked	1
WP3-3	IMEP32-5 fish fillet spiked	2.5
WP3-4	Blue mussel powder	0.3
WP3-5	Crab powder	0.1
WP3-6	DORM-3 Dogfish muscle	0.2
WP3-7	TORT-2 Lobster Hepatopancreas	0.8

- 10 labs (one lab gave 2 sets of results => 11 datasets)
- SPE separation procedure was followed
- Both HG-AAS and ICPMS were used for determination of iAs

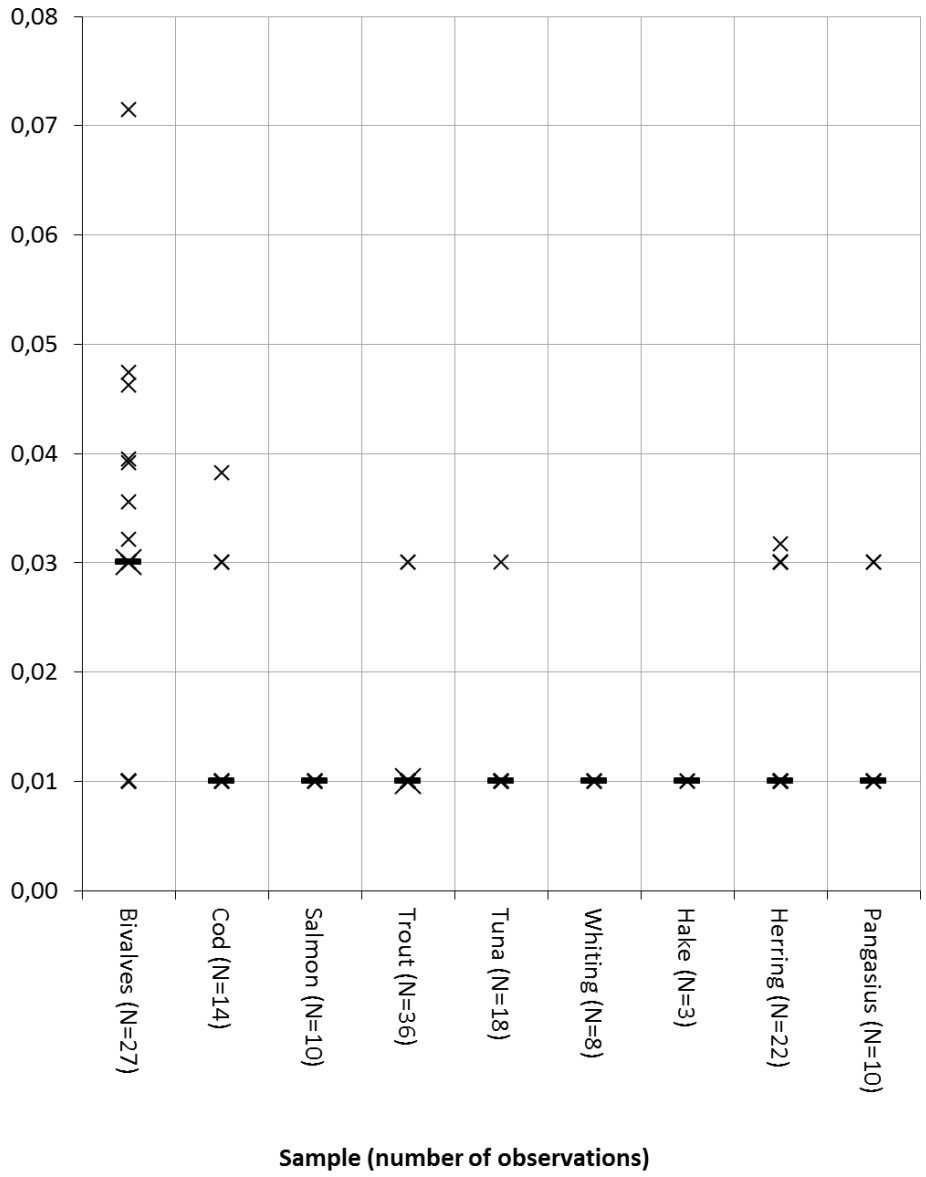
Collaborative trial – marine samples

	Unit	WP3-2	WP3-3	WP3-4	WP3-5	WP3-6	WP3-7
No of labs		11	11	11	11	11	11
No of non-compliant labs		3	2	6	1	1	3
No of compliant labs		8	9	5	10	10	8
Overall mean	mg kg^{-1}	1,03	2,57	0,26	0,14	0,19	0,76
					Low conc		
S_r	mg kg^{-1}	0,12	0,20	0,04	0,03	0,02	0,06
RSD_r	%	11,5	7,9	14,1	23,2	13,1	7,6
r_L	mg kg^{-1}	0,33	0,57	0,10	0,09	0,07	0,16
S_R	mg kg^{-1}	0,17	0,34	0,07	0,09	0,04	0,13
RSD_R	%	16,5	13,4	26,7	64,1	22,1	17,4
R_L	mg kg^{-1}	0,47	0,96	0,19	0,26	0,12	0,37
Horwitz value		15,8	13,8	19,5	21,3	20,4	16,6
HorRat		1,0	1,0	1,4	3,0	1,1	1,1

- Precision: RSD_r : 8 - 14% and RSD_R :13 - 27%
- Accuracy: 89-100%
- Measurement range: 0.2 - 2.6 mg/kg
- HorRat: 1.0 – 1.4
- HG-AAS vs ICPMS: no difference

- Blue mussel sample (WP3-4): not satisfactory results

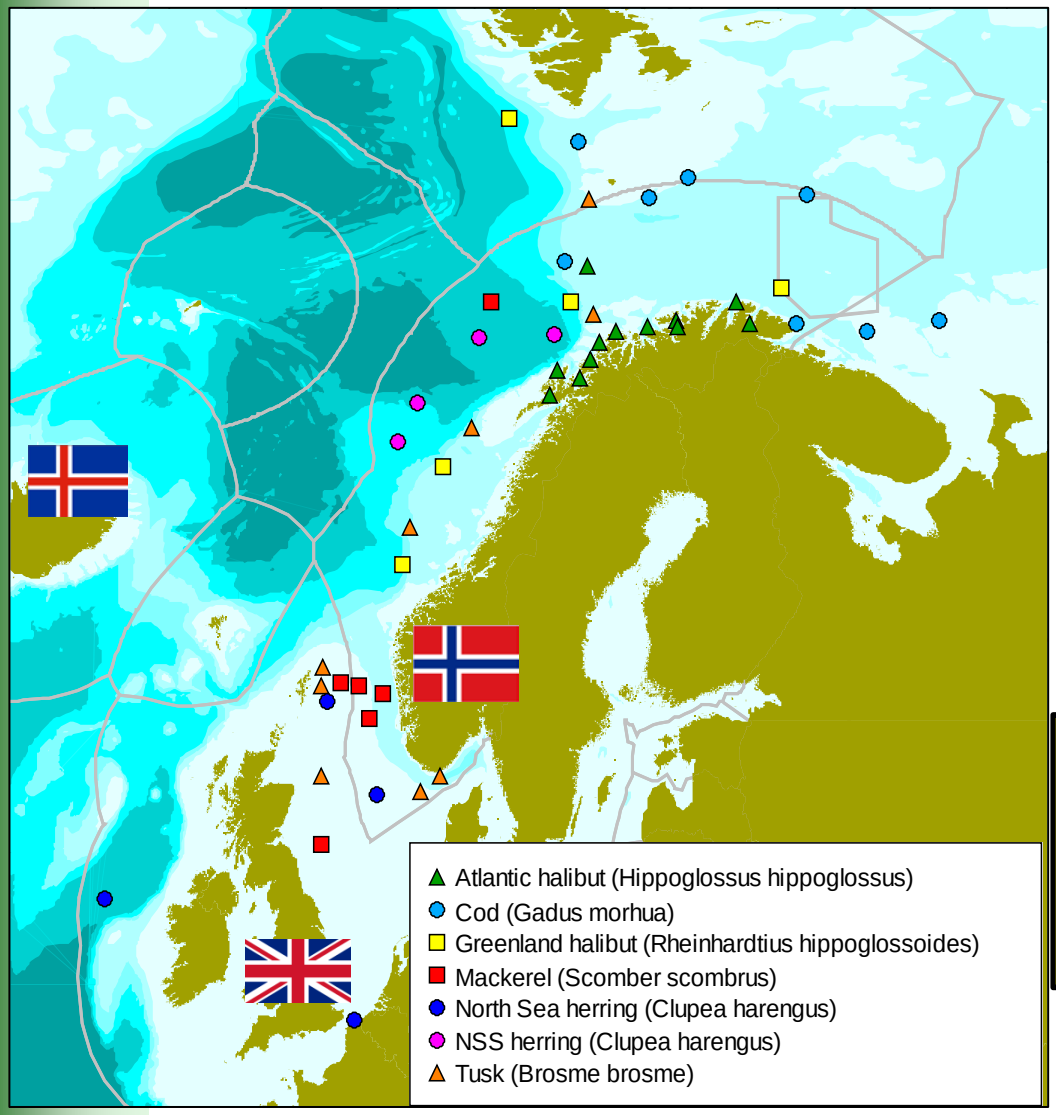
Survey data – marine samples



Inorganic arsenic

- 148 seafood samples
- all fish <0.04 mg/kg
- bivalves <0.01 – 0.07 mg/kg

Inorganic arsenic in wild caught fish => no concern



Norwegian survey

900 individual fish samples

- Atlantic halibut
- Cod
- Greenland halibut
- Mackerel
- Herring
- Tusk

Results

Total arsenic…….0.3-110 mg/kg

Inorganic arsenic…. < 0.01 mg/kg

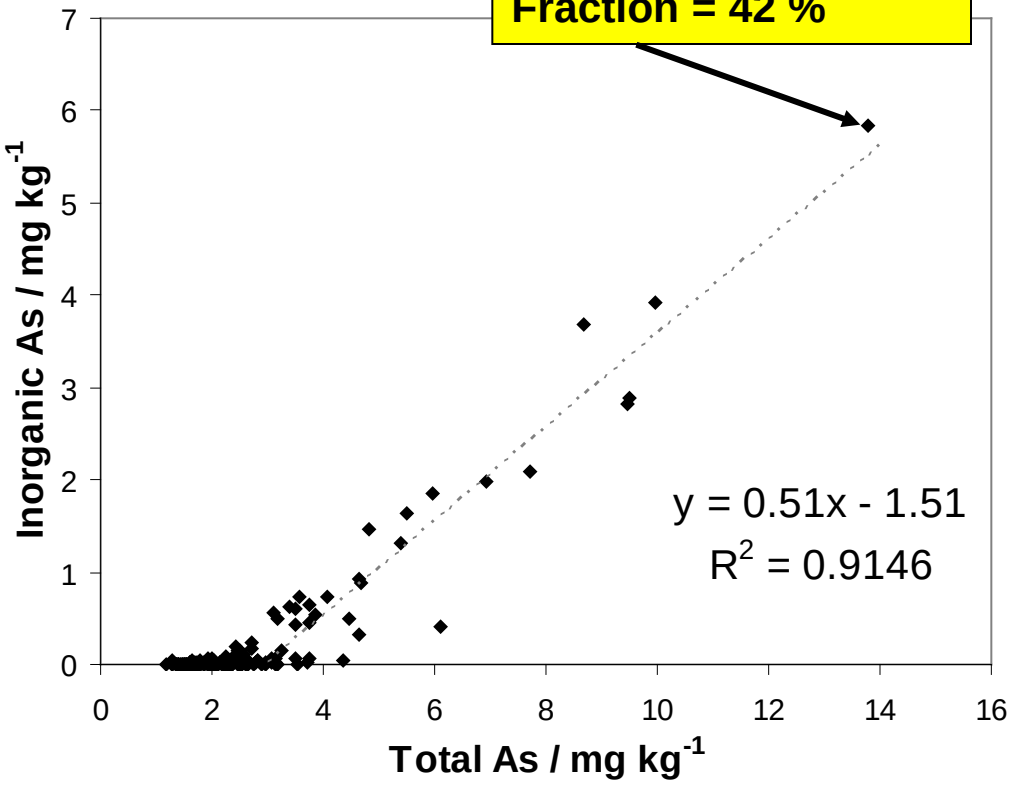
(only 37 samples > LOQ)

NIFES NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH

Julshamn and Sloth, Fd Addit Contam B, 2012, in press

...but in bivalves high contents in some samples...

Total As = 13.8 mg/kg
Inorg As = 5.8 mg/kg
Fraction = 42 %



Data from 175 blue mussel (*Mytilus edulis*) samples collected along the Norwegian Coastline.

Sloth and Julshamn, 2008, J. Agri.Food Chem., 56, 1269-1273

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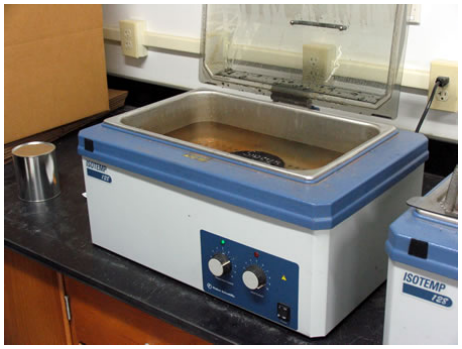
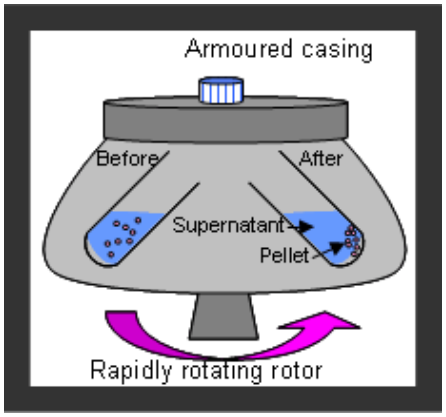
SPE HG-AAS – iAs in rice

Sample + 10 mL extractant
(0,1 M HNO_3, 3% H_2O_2)

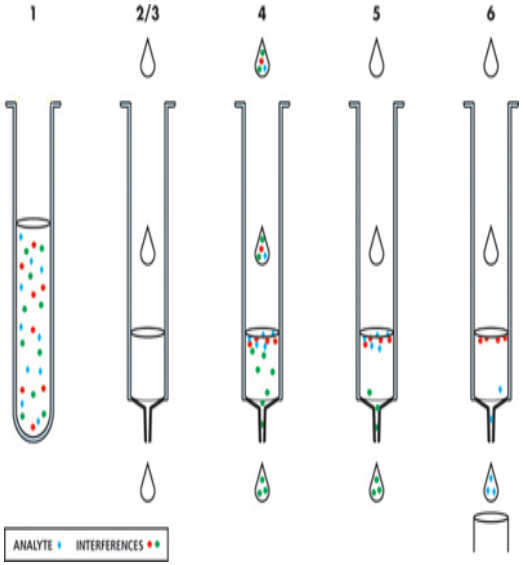


90°C waterbath,1h



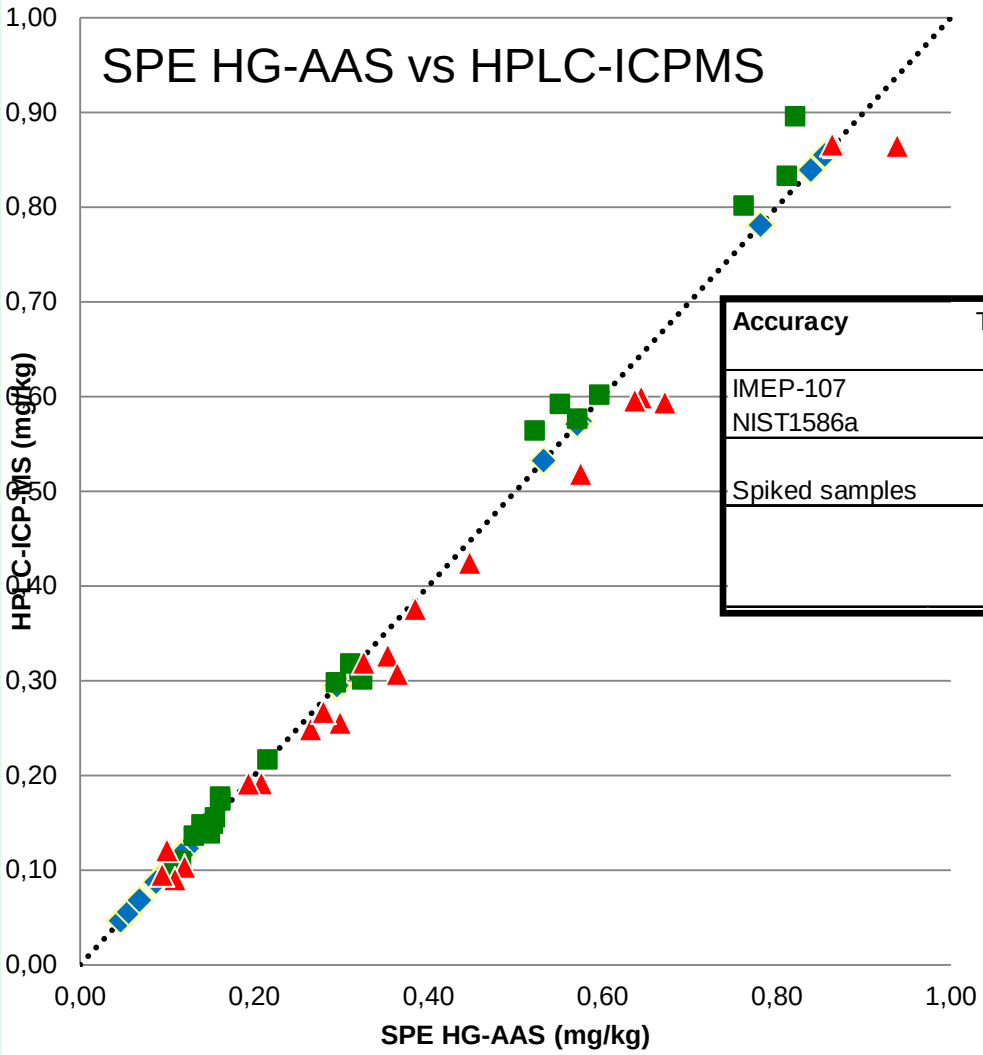
centrifugation

SPE separation



HG-AAS

SPE HG-AAS – iAs in rice - validation



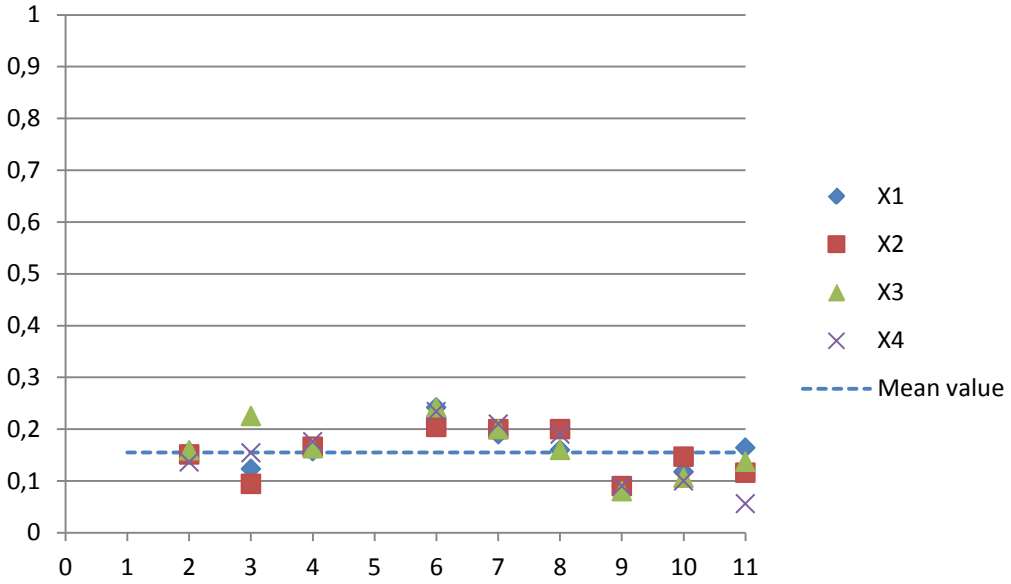
Accuracy	Target value (mg/kg)	Found (mg/kg) mean +/- 2s	
IMEP-107	0,107 +/- 0,014	0,108 +/- 0,017	(N=6)
NIST1586a	0,097	0,101 +/- 0,014	(N=6)

Spiked samples	Spike level	Recovery	
	0,30 mg/kg	105 %	(N=9)
	0,55 mg/kg	106 %	(N=9)
	0,80 mg/kg	106 %	(N=9)

Precision		
Repeatability	RSDr	4,8 %
Reproducibility	RSDR	7,8 %

LoD / LoQ		
LoD	(k=3)	0,02 mg/kg
LoQ	(k=6)	0,04 mg/kg

SPE HG-AAS – iAs in rice – collaborative study



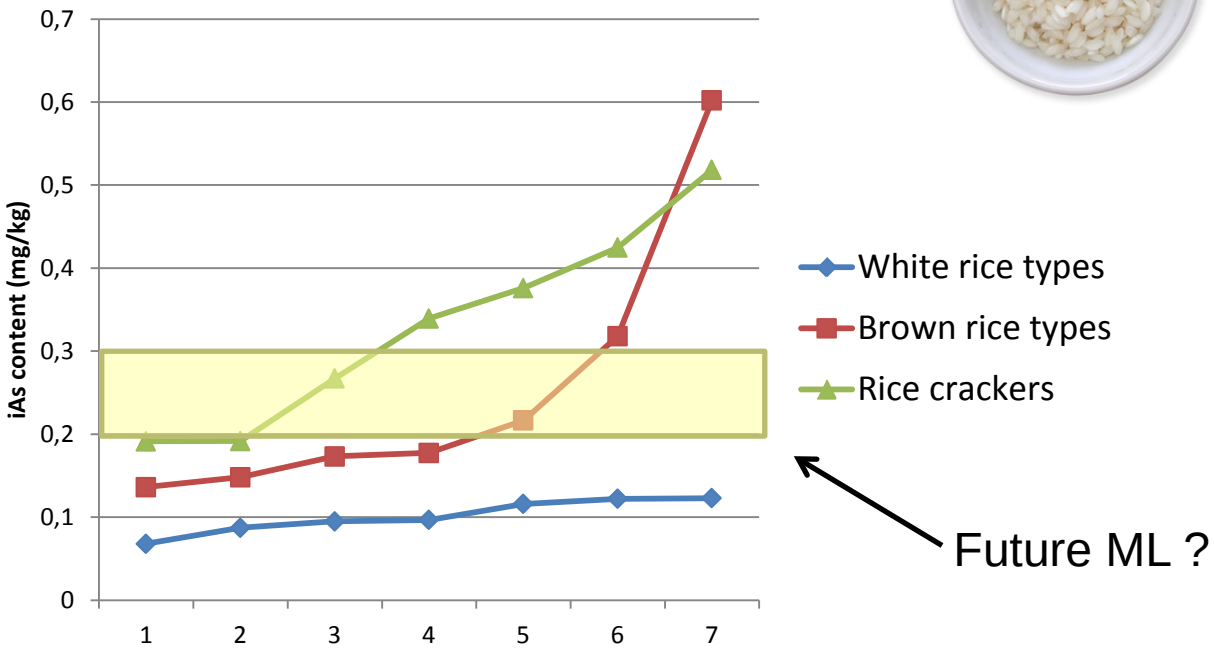
	WP3-9
No of labs	11
No of non-compliant labs	2
No of compliant labs	9
Overall mean	0,16
S_r	0,03
RSD_r	18,3
r_L	0,08
S_R	0,05
RSD_R	30,0
R_L	0,13
Horwitz value	21
HorRat	1,4

Test sample:
Wholemeal rice flour
(organic)

Survey data – iAs in rice samples

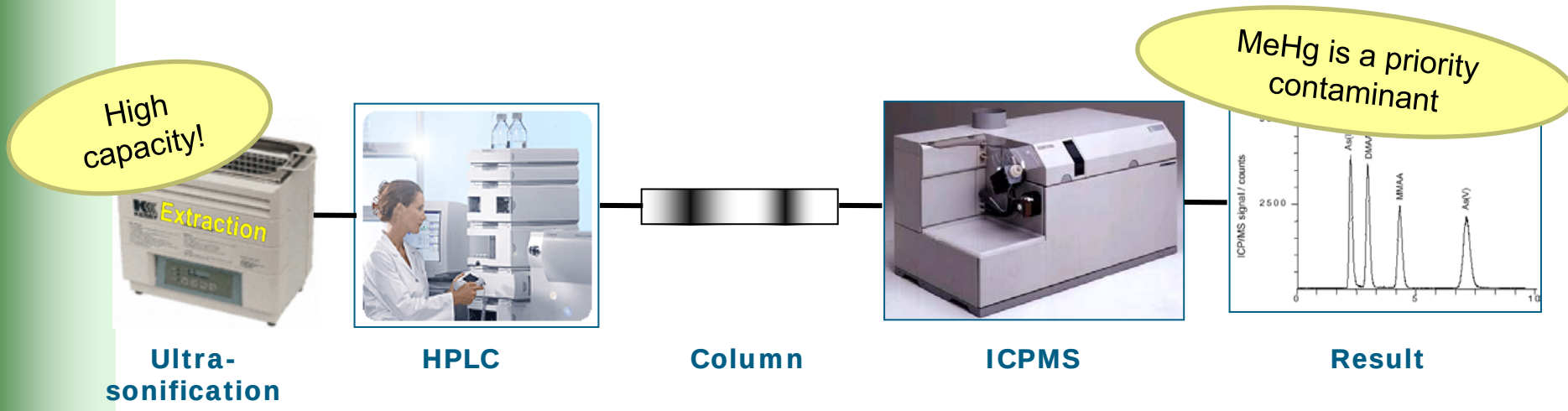
21 samples (so far)

- White rice
- Brown rice types
- Rice crackers



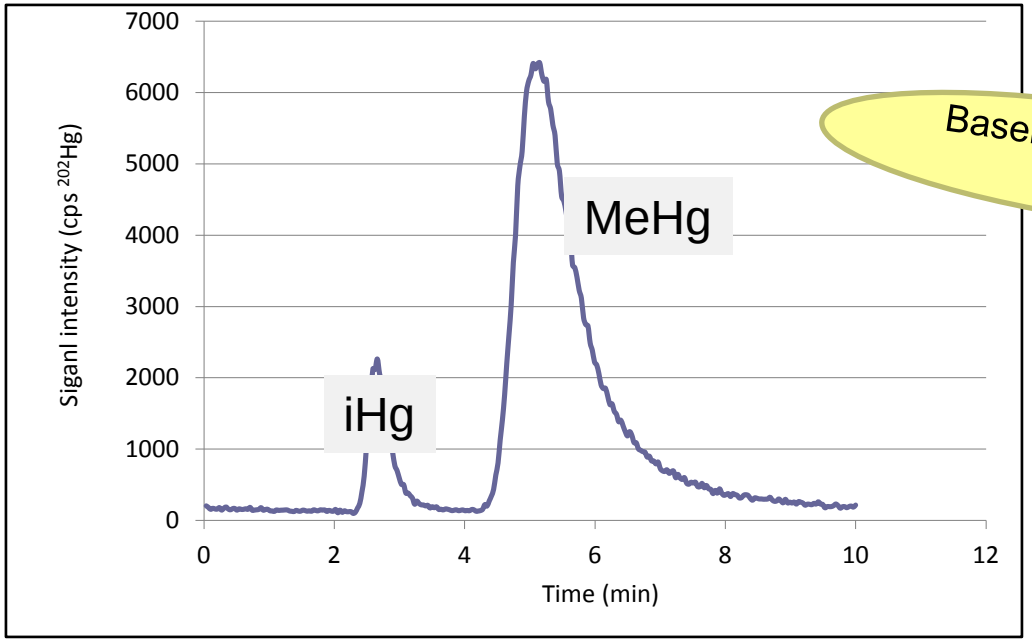
Future ML ?

Speciation analysis of mercury by HPLC-ICPMS



- 0.5 gram sample (2 x extraction with 5 ml 5 M HCl)
- Centrifugation
- pH adjustment
- Cation exchange (Hamilton PRP X200 SCX)
- HPLC-ICPMS

Cation exchange HPLC-ICPMS



HPLC-ICPMS chromatogram of DORM-3 (Dogfish muscle)

Performance of the HPLC-ICP-MS method for determination of MeHg

	DORM-2 *Dogfish*	TORT-2 *Lobster*	DORM-3 *Dogfish*	Fishfeed #1	Fishfeed#2	Codfish	Salmon
Ref level (mg/kg)	4.47	0.15	0.36	0.21	0.06	0.17	0.06
Observations (N)	9	15	9	9	9	9	9
Mean recovery (%)	94	102	96	-	-	-	-
Repeatability RSD_r (%)	3	4	3	11	13	5	13
Reproducibility RSD_{IR} (%)	8	12	8	11	15	12	20
Horwitz Rel. Std. (%)	13	21	19	20	25	21	25

Setup

- Natural incurred samples
  - CRMs (DORM-2, DORM-3 and TORT-2)
  - fish feed, codfish and salmon
- Analysed in triplicates on 3 different days
- 2 technicians

Results overview

- 0.004 mg/kg limit of detection (LOD)
- Mean repeatability = 7%
- Reproducibility < Horwitz RSD
- 94-102% recovery

Collaborative trial – marine samples

- Small scale ILC (4 labs)
- 6 samples (0,15 – 5,5 mg/kg)
- Both seafood and feed

		Target value	LAB1	LAB2	LAB3	LAB4
WP3-1	Complete feed (spiked)	0,19	0,21	0,20	Data to be produced Sept/Oct	
WP3-3	Fish fillet (spiked)	1,8	2,08	1,91		
WP3-5	Crab powder	0,28	0,35	0,34		
WP3-6	DORM-3	0,355	0,38	0,34		
WP3-7	TORT-2	0,152	0,17	0,15		
WP3-8	CE464 Tunafish	5,5	5,53	5,61		

Survey data - MeHg in fish feed and ingredients

Type	Sample ID	% Fat	Hg (total) (µg/kg)	MeHg (µg/kg)
Fish silage	204557	11.8	39	<30
	205398	11.3	40	<30
	207967	10.7	39	<30
	207976	9.2	11	<30
	208547	11.3	55	<30
Fish oil	201224	100	<10	na
	201225	100	<10	na
	205376	100	<10	na
Complete feed	207847	34.6	24	<30
	210554	28.8	18	<30
	210555	17.0	36	<30
	210606	24.8	49	32
Fish meal	201226	13.7	120	125
	201227	14.0	93	79
	202128	13.7	71	45
	202141	8.2	48	30
	204687	12.0	30	<30
	204836	10.3	43	<30
	206945	10.4	34	<30
	207833	12.0	33	<30
	207899	12.3	27	<30
	210705	11.0	69	53
	211035	6.0	67	55
	211612	7.9	40	<30
	211662	14.4	61	53
	211669	9.7	44	32

All samples collected as part of the national surveillance/feed-control programme in Denmark

EU maximum level

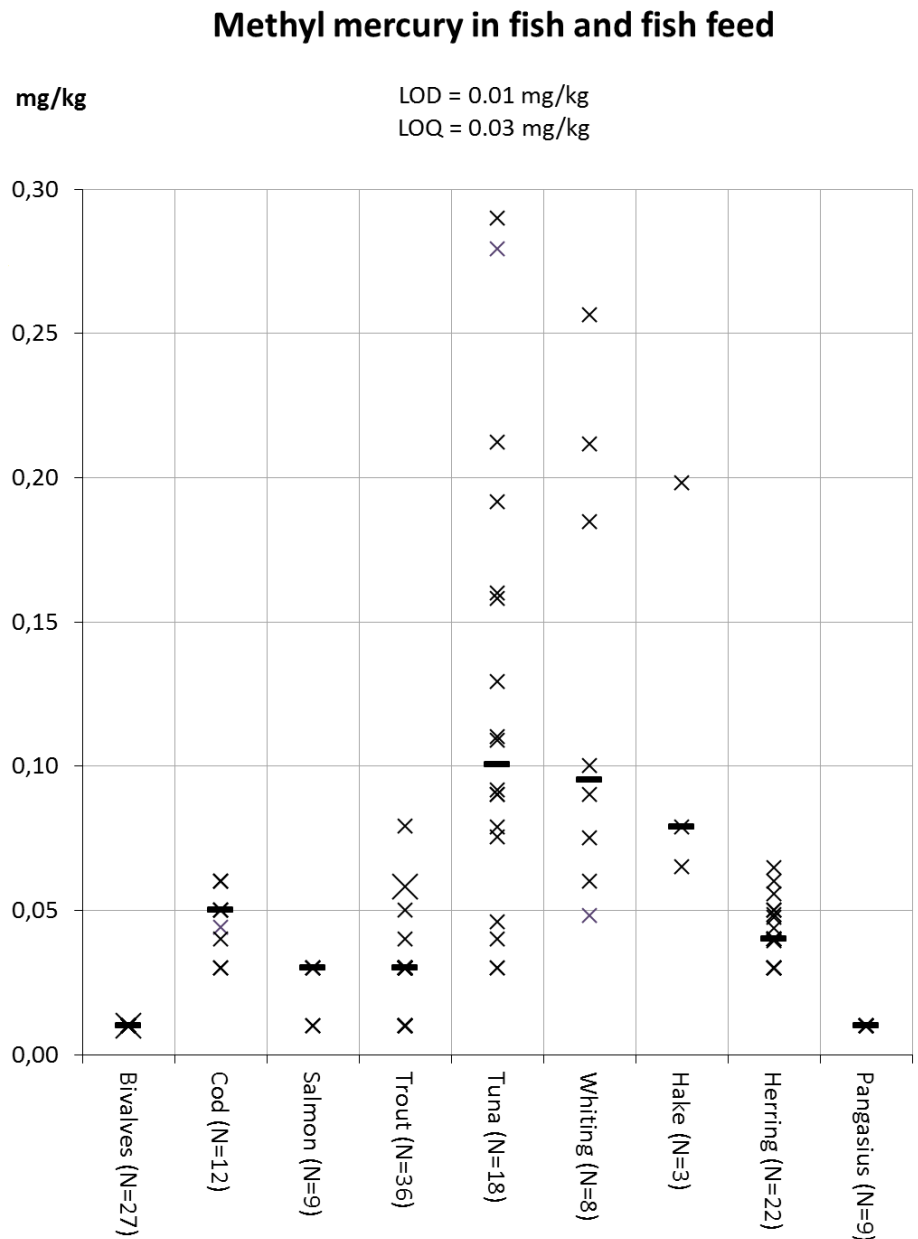
-No ML for MeHg

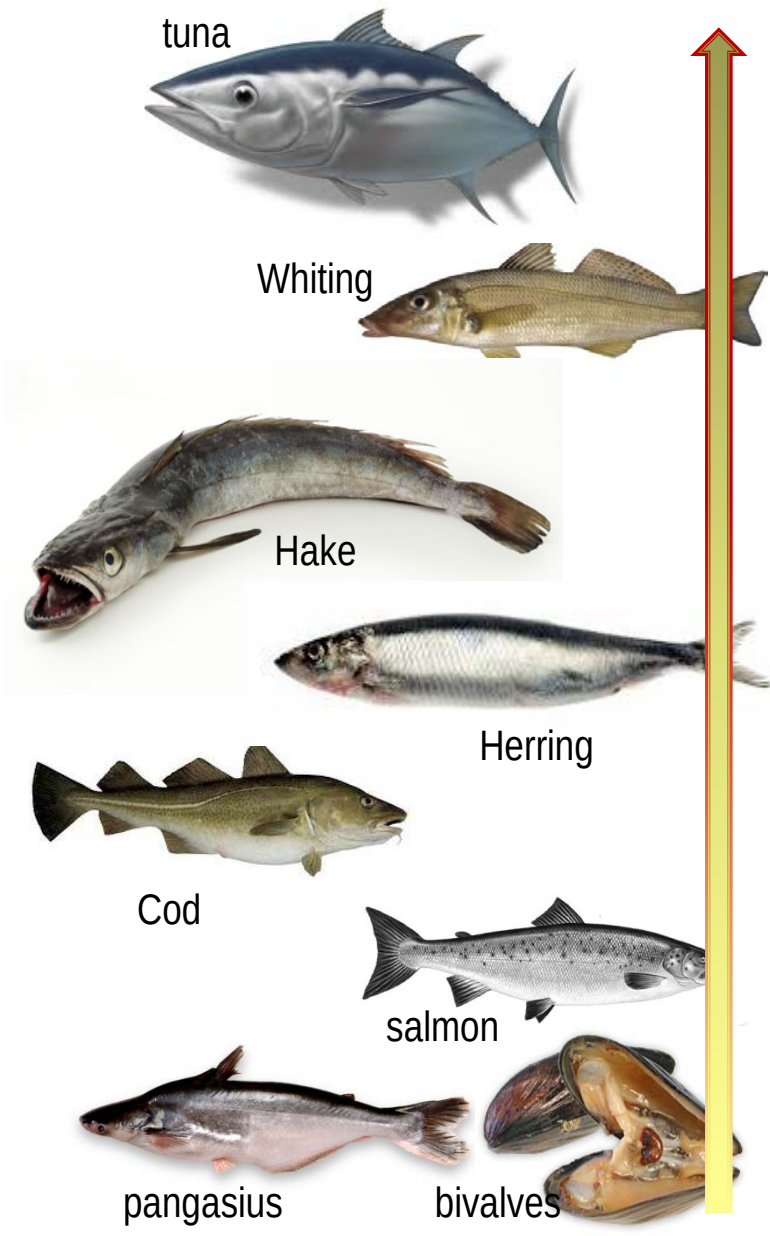
- 0.2 mg/kg for total Hg *(2010)*

(before 2010 the ML= 0.1 mg/kg)

-all samples < ML

Survey data – MeHg in seafood





Output from CONffIDENCE WP3

Methods:

- iAs in marine samples by SPE HG-AAS
- iAs in rice samples by SPE HG-AAS
- MeHg in marine samples by HPLC-ICPMS

Collaborative trials:

- iAs in marine samples by SPE HG-AAS (10 labs)
- MeHg in marine samples by HPLC-ICPMS (4 labs)
- ”target values” established for future QA purposes

Survey data:

- iAs in marine samples (N=130)
- iAs in rice samples (N=30)
- MeHg in marine samples (N=130)

Contribution to risk-benefit analysis :

- Seafood samples analysed for POPs and fatty acids (with WP1)
- Reported to EFSA databases for future risk evaluations

Further information

- www.conffidence.eu
- CONffIDENCE newsletters
- Scientific publications

- Hedegaard and Sloth, Heavy metal speciation in feed: why and how?, BASE, 2011, 15, 45-51.
- Rasmussen *et al*, Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin, Anal Bioanal Chem, 2012, 403, 2825-2834.
- Rasmussen et al, Development and validation of a HPLC-ICPMS method for determination of methylmercury in marine food and feed, Anal Bioanal Chem (CONffIDENCE special issue), *in prep* (expected 2013)
- Sloth et al, Contaminant and fatty acid profiles in European seafood, *in prep* (expected 2013)

- Contact: Jens J. Sloth (jjsl@food.dtu.dk) (WP3 leader)

- Thanks for your attention!

European Commission

Joint Research Centre (JRC)

Serving society, stimulating innovation, supporting legislation

"IMEP-115: Methylmercury in seafood – A Collaborative trial"

Dr. Fernando Cordeiro
Institute for Reference Materials and Measurements, Geel, Belgium.

(fernando.cordeiro-raposo@ec.europa.eu)

Joint Research Centre

7th EU-RL-HM Workshop, 20th Sept. 2012

European Commission

Support Commission Regulation 1881/2006 (max. levels of contaminants in foodstuffs)

Methyl mercury determination based on a double liquid-liquid extraction, firstly with organic solvent and subsequently with a cysteine solution.

Measurement with an elemental mercury analyser – Protocol provided!

Joint Research Centre

Test material	X_{ref}	U_{ref} (k=2)
Material 1	1.33	0.12
Material 2	0.152	0.013
Material 3	0.069	0.0008
Material 4	0.0132	0.0007
Material 5	5.50	0.34

7th EU-RL-HM Workshop, 20th Sept. 2012

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Scrutinize the data following ISO 5275-2:1994

$$C = \frac{S^2_{\max}}{\sum_{i=1}^{p} S_i^2}$$ **Cochran test**

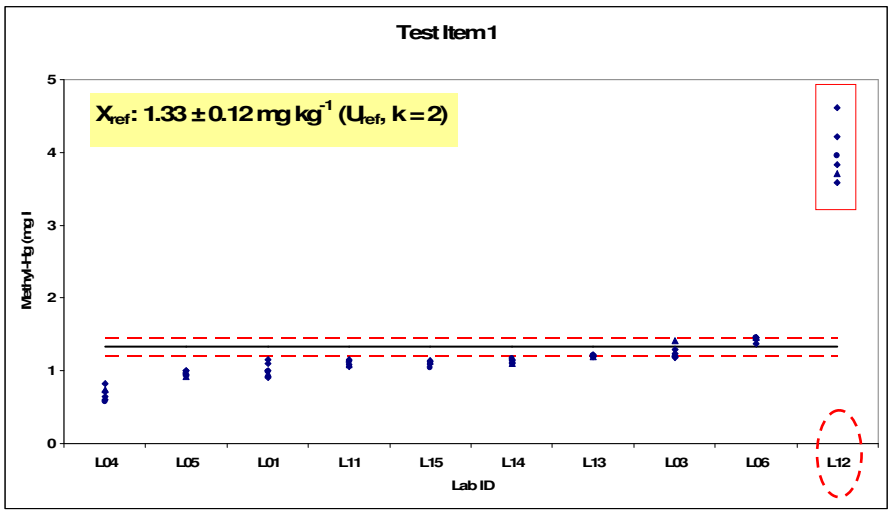
$$G_p = \left(X_p - \overline{X}\right) / S$$ **Grubbs (Higher)**

$$G_l = \left(\overline{X} - X_l\right) / S$$ **Grubbs (Lower)**

Joint Research Centre

European Commission

Test Item 1



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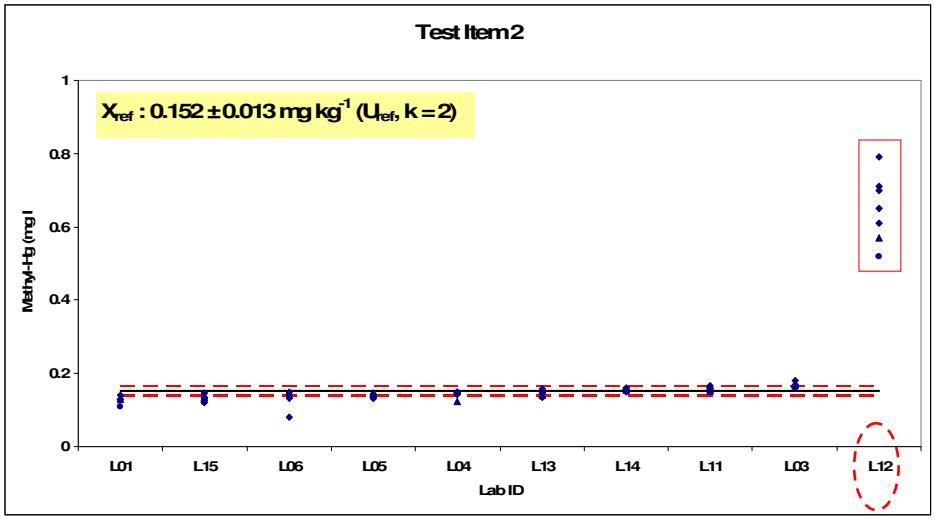
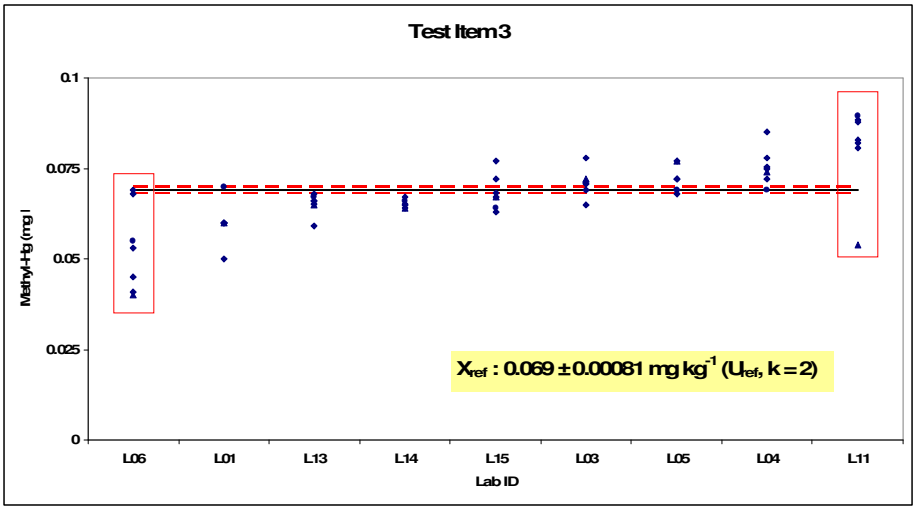
European Commission

Test Item 2



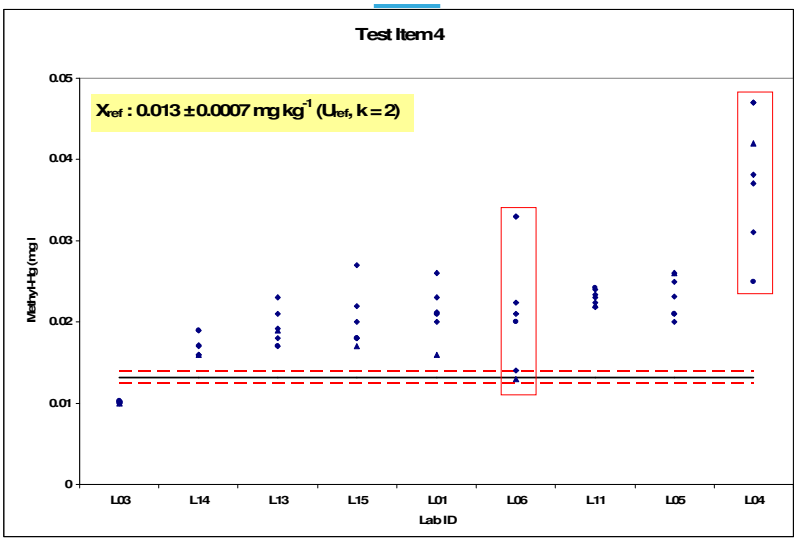
Joint Research Centre

European Commission

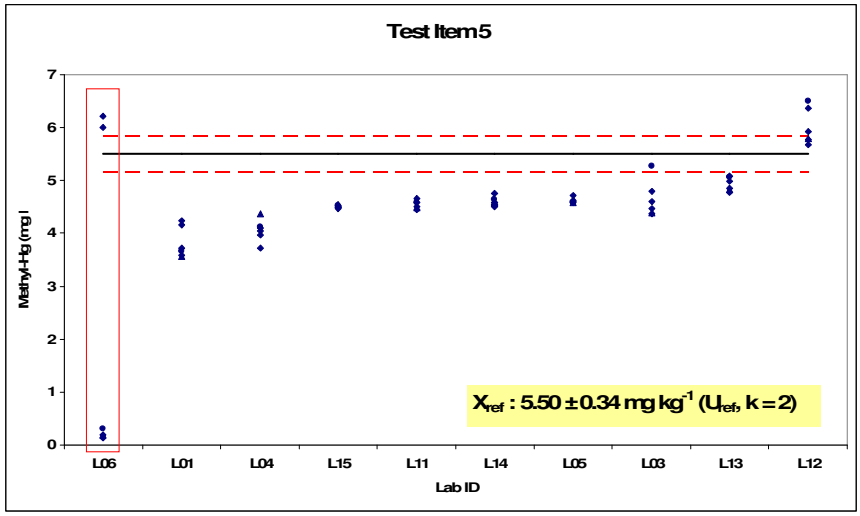


Joint Research Centre

European Commission



Joint Research Centre



Establish the method precision:

Repeatability Std: S_r *and* r

Between-lab Std: S_{Be} *and* R

Reproducibility Std: $S_R = \sqrt{(S^2_r + S^2_{Be})}$

Recovery: X_{mean}/X_{ref} *(%)*

European Commission

Test Item 1 (after excluding L12)

LCode	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Mean
L04	0.61	0.65	0.74	0.71	0.83	0.58	0.687
L05	1	1	0.92	0.95	0.94	0.96	0.962
L01	1.16	1.1	0.96	0.9	1	0.99	1.018
L11	1.082	1.134	1.079	1.06	1.15	1.11	1.103
L15	1.12	1.1	1.13	1.14	1.13	1.05	1.112
L14	1.101	1.113	1.1	1.157	1.141	1.162	1.129
L13	1.19	1.21	1.19	1.19	1.22	1.21	1.202
L03	1.23	1.29	1.42	1.18	1.19	1.19	1.250
L06	1.45	1.45	1.46	1.43	1.37	1.45	1.435

Mean of L04 is NOT an outlier (Grubbs)

Preliminary results!

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Test Item 1 (after excluding L12)

X_{ref}: 1.33 ± 0.12 mg kg^{-1} (U, k=2)

S_r =	0.062			
S_{Be} =	0.174			
S_R =	0.185			
r =	0.17	mg Kg^{-1}		
R =	0.52	mg Kg^{-1}		
X =	1.10	mg Kg^{-1}	(*10^{-6})	
RSD_r =		5.6	%	
RSD_R =		16.8	%	
σ_H =		1.73E-07	0.173	mg Kg^{-1}
$PRSD_R$ =		15.8	%	
HorRat =		1.06		
Rec (%) =	82.7	$(R_{uObs})^2$ =	0.028164	
		$(Ru_{Ref})^2$ =	0.002035	
		u_{Rec} =	14.4	

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European Commission

Test Item 5 (after excluding L06)

X_{ref}: 5.50 ± 0.34 mg kg^{-1} (U, k=2)

S_r =	0.212			
S_{Be} =	0.605			
S_R =	0.641			
r =	0.59	mg Kg^{-1}		
R =	1.79	mg Kg^{-1}		
X =	4.64	mg Kg^{-1}	(*10^{-6})	
RSD_r =		4.6	%	
RSD_R =		13.8	%	
σ_H =		5.89E-07		0.589 mg Kg^{-1}
$PRSD_R$ =		12.7	%	
HorRat =		1.09		
Rec (%) =	84.3	$(R_{uObs})^2$ =	0.0191026	
		$(Ru_{Ref})^2$ =	0.0009554	
		u_{Rec} =	11.9	

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Is the method adequately precise?

Compare precision characteristics with literature and SOP acceptance criteria…

Journal of the Science of Food and Agriculture, 88:2543-2550 (2008)

Muscle: 84 ± 6 mg kg^{-1} (SD, n ≥ 4) RSD 7.0 %

Liver: 75 ± 8 mg kg^{-1} (SD, n ≥ 4) RSD 11 %

Method seems adequately precise !!

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Is the method adequately accurate?

Compare precision characteristics with CRM documentation …

Results from the stability monitoring range from 5.17 to 5.72 mg kg^{-1}

Analytical recovery < 85 % (85–115 %)!!

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Preliminary results!!

At least 1-2 labs missing !!

Discuss and identify reasons for lower recovery !!

Joint Research Centre

IMEP-114 (& IMEP-36): Determination of total Cd, Pb, As, Hg and Sn in feed premixes

Dr. Ioannis Fiamegkos

European Commission, Directorate General Joint Research Centre, Institute for Reference Materials and Measurements, B-2440 Geel, Belgium

24 October 2012

Samples

Reference values

Participants

Measurement results

Test item

- Commercially available feed premix


426 /000
PROLACTA - MIN.MENGSEL VR DROOGST.KOEIEN
PROLACTA - ALIM.MINERAL PR VACHES TARIES
L/1.26012012.8410
Ten minste houdbaar tot : 26-01-2013
A utiliser de préférence avant :
5 411866
Mineraalvoeder voor droogstaande koeien.
Aliment minéral pour vaches taries.
INHOUD - CONTENU: 25 kg
ERKENNING FABRIKANT /AGRÉMENT FABRICANT:
GEBRUIKSAANWIJZING/MODE D'EMPLOI
Koel en droog bewaren./A conserver dans un endroit frais et sec.
250 g per dier per dag:
(1) Genetisch gemodificeerde soja.
250 g par animal par jour.
(1) Soja génétiquement modifié.
ANALYTISCHE BESTANDDELEN - CONSTITUANTS ANALYTIQUES
Calcium 0.22 %; Totale fosfor/Phosphore totale 3.95 %; Magnesium/Magnésium 8.00 %; Natrium/Sodium 3.01 %.
TOEVOEGINGSMIDDELEN/ADDITIFS
Nutritionele toevoegingsmiddelen: /Additifs nutritionnels:Vitamine A (E672) 1000000 IE/kg/UI/kg; Vitamine D3 (E671) 200000 IE/kg/UI/kg; Vitamine E (all-rac-alfa-tocoferyl acetaat) (3a700)/Vitamine E (all-rac-alfa-acétate de tocophéryl) (3a700) 4400 mg/kg; Cholinechloride 2000 mg/kg; Watervrij calciumjodaat, Jodium (E2)/Iodate de calcium, anhydre, Iode (E2) 20.0 mg/kg; Kobalt(II)sulfaat, heptahydraat, Kobalt (E3)/Sulfate de Cobalt, heptahydrate, Cobalt (E3) 15.00 mg/kg; Koper(II)sulfaat, pentahydraat, Koper (E4)/Sulfate cuivrique, pentahydraté, Cuivre (E4) 1000 mg/kg; Mangaan(II)oxide, Mangaan (E5) /Oxyde manganeux, Manganèse (E5) 1250 mg/kg; Zinksulfaat, monohydraat, Zinc (E6)/Sulphate de zinc, monohydraté, Zinc (E6) 2500 mg/kg; Natriumseleniet, Selenium (E8)/Sélénite de Sodium, Sélénium (E8) 40.00 mg/kg
Technologische toevoegingsmiddelen:/Additifs technologiques:BHT (E321) 100.00 mg/kg
SAMENSTELLING - COMPOSITION
d'extraction de tournesol, Chlorure de sodium, Mélasse de betterave, Oxyde de magnésium, Huile de palme, Graines de soja cuites (1).

Test item

- Commercially available feed premix
- The feed pre-mix pellets were milled and homogenised
- 20 g of feed material used to fill 60 mL powder bottles
- Samples dispatched on the 27th of June
- The deadline for submitting the results was the 7th of September
- The measurement results were to be corrected for (i) recovery and (ii) moisture, following the procedure described therein
- The homogeneity and stability studies were undertaken by ALS Scandinavia AB (Sweden)
- **The Federal Institute for Materials Research and Testing (BAM) and LGC Limited were employed for delivering the assigned values.**

Analytes

- Total Cd, Pb, As, Hg and Sn

European Commission

Samples

Reference values

Participants

Measurement results

Joint Research Centre

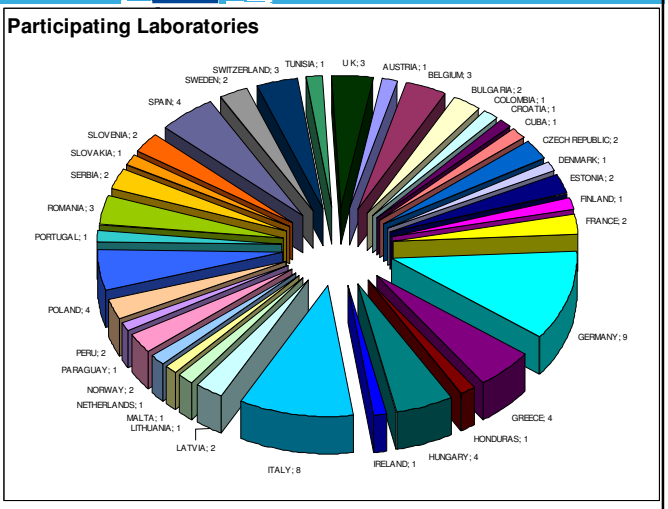
European Commission

IMEP-114

- Registered 30 NRLs from 26 countries

Reported results by NRLs:

- 25 for total As,
- 30 for total Cd,
- 29 for total Pb,
- 13 for total Hg and
- 9 for total Sn



For both IMEP-114 and IMEP-36:

- Registered 80 laboratories from 35 countries

Joint Research Centre

European Commission

Samples

Reference values

Participants

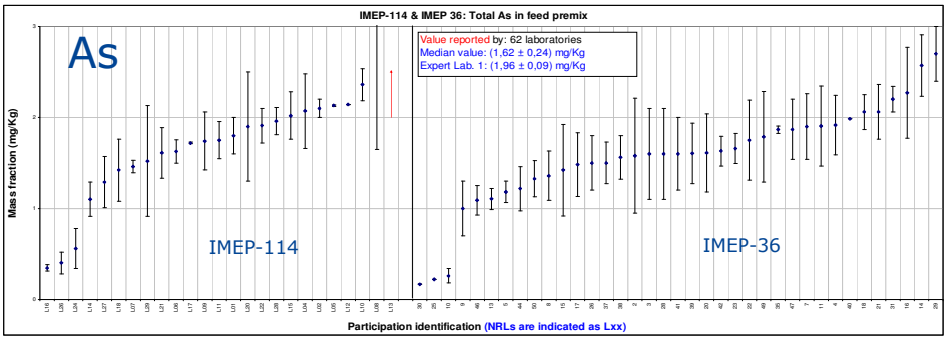
Measurement results

Joint Research Centre

European Commission

Total As:

- Values reported by 25 participants of IMEP-114 and 37 of IMEP-36



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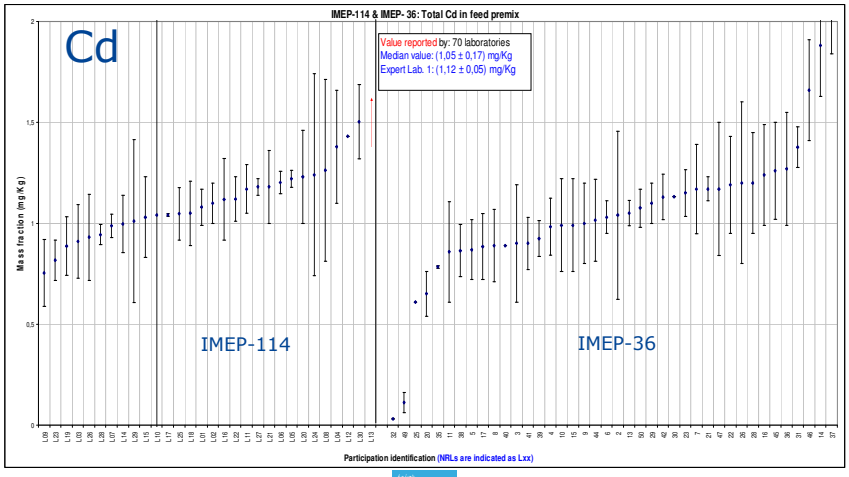
Total Cd:

- Values reported by 30 participants of IMEP-114 and 40 of IMEP-36



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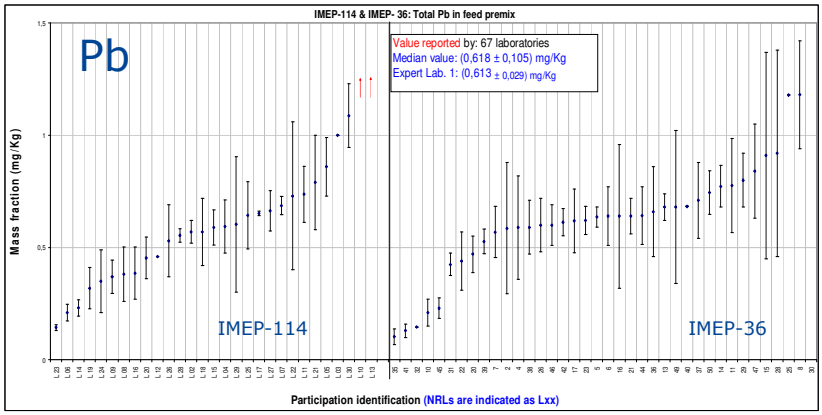
Total Pb:

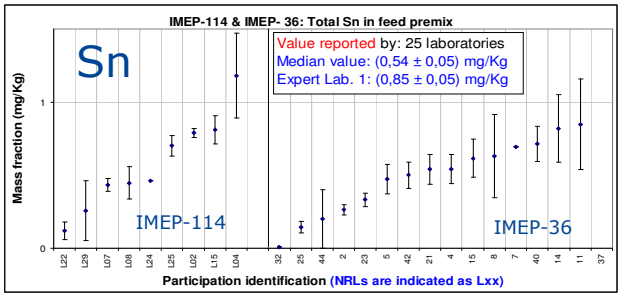
- Values reported by 29 participants of IMEP-114 and 38 of IMEP-36



Joint Research Centre

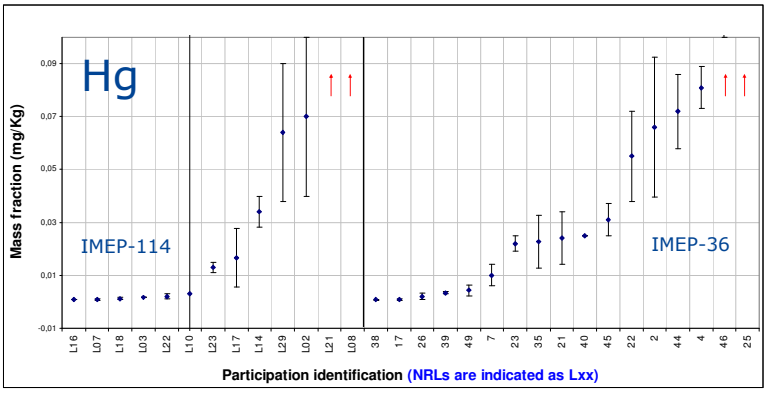
Total Sn:

- Values reported by 9 participants of IMEP-114 and 16 of IMEP-36



Total Hg:

- Values reported by 13 participants of IMEP-114 and 17 of IMEP-36
- "Less than" was reported by 10 participants of IMEP-114, 11 of IMEP-36 and by the Expert Lab. 1



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- Let's discuss…..

Lab ID	Comments
L02	Results were not corrected for recovery, because we do not do it in routine sample analysis.
L03	Our laboratory is accredited for Hg analysis and Cd/Pb method is under final validation.
L08	This Laboratory not yet accredited for Tin analyses. It is not accredited for Feed Premixes, only Food matrices. Analytical problems were encountered because of high quantity of insoluble matter in the acid digested sample which resulted in further difficulties with Arsenic and Mercury analyses.
L08	This Laboratory not yet accredited for Tin analyses. It is not accredited for Feed Premixes, only Food matrices. Analytical problems were encountered because of high quantity of insoluble matter in the acid digested sample which resulted in further difficulties with Arsenic and Mercury analyses.
L12	Pb, Cd and As is not accredited: validation in progress
L15	We don´t correct the results for recovery
L18	The method we used is only validated and used on a regular basis for food matrices. This SOP is not validated for feed
L20	We have send the material to a subcontracter for analysis of As, Cd, Pb and Hg. The aim was to check the subcontracter.
L22	Determination of Sn is not accredited because our laboratory do not provide this deteremination in feed.
L28	Our laboratory is accredited for food matrix, we don't analysis feed matrix

European Commission
JRC 76140 – Joint Research Centre – Institute for Reference Materials and Measurements

Title: 7th Workshop of the European Reference Laboratory for Heavy Metals in Feed and Food

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Abstract

The task of the EU-RL for Heavy Metals in Feed and Food is to facilitate the implementation of Regulation (EC) N° 1881/2006 and Directive 2001/22/EC establishing the maximum levels of heavy metals such as lead, mercury and cadmium in different foods and feed.

One of the duties of the EU-RL-HM is to organise a workshop for the network of National Reference Laboratories and to report on main subjects dealt with in the mentioned workshop.

This report summarises the discussions that took place during the 7th Workshop organised by the EU-RL-HM which took place in Brussels on the 20th September 2012 and the agreements reached on that occasion.

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

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Key policy areas include: environment and climate change; energy and transport; agriculture and food security; health and consumer protection; information society and digital agenda; safety and security including nuclear; all supported through a cross-cutting and multi-disciplinary approach.