Contaminants

Annual report 2012 of the National Reference Laboratory

W.A. Traag, J.G.J. Mol, M.K. van der Lee, A. Gerssen, M.Y. Noordam and L.A.P. Hoogenboom

RIKILT
WAGENINGEN UR

Contaminants

Annual report 2012 of the National Reference Laboratory

W.A. Traag, J.G.J. Mol, M.K. van der Lee, A. Gerssen, M.Y. Noordam and L.A.P. Hoogenboom

This research was (partly) funded by the Dutch Ministry of Economic Affairs (EZ), WOT 02-Food Safety, Theme 1 Contaminants.

RIKILT Wageningen UR
Wageningen, June 2013

RIKILT report 2013.007

RIKILT
WAGENINGEN UR

Traag, W.A., J.G.J. Mol, M.K. van der Lee, A. Gerssen, M.Y. Noordam and L.A.P. Hoogenboom, 2013. *Contaminants; Annual Report 2012 of the National Reference Laboratory.* Wageningen, RIKILT Wageningen UR (University & Research centre), RIKILT report 2013.007. 48 pp.;6 tab.; 48 ref.

Project number: 72311, 72314, 72316, 72312, 72315, 72683
BAS-code: WOT-02-001-004, WOT-02-001-005, WOT-02-001-006, WOT-02-001-007, WOT-02-001-008, WOT-02-001-010
Project title: National Reference Laboratory dioxins and PCBs, pesticides in animal derived products, mycotoxins, heavy metals, polycyclic aromatic hydrocarbons, and marine toxins
Project leader: W.A. Traag, J.G.J. Mol, M.K. van der Lee, and A. Gerssen

P.O. Box 230, 6700 AA Wageningen, The Netherlands, T +31 (0)317 48 02 56, E info.RIKILT@wur.nl, www.wageningenUR/nl/en/rikilt. RIKILT is part of Wageningen UR (University & Research centre).

RIKILT report 2013.007

Distribution list:

- Ministry of Economic Affairs (EZ); E.R. Deckers, I.R.J. van de Pas.
- Netherlands Food and Consumer Product Safety Authority (NVWA); R.M.C. Theelen, J.A. van Rhijn, A. de Kok, R.G. Herbes, G.A. Lam, J.H. Jeuring, W.A. de Leeuw, R. Dwinger.
- Institute for Reference Materials and Measurements (JRC-IRMM, Geel, Belgium); T. Wenzel, B. De la Calle, J. Stroka.
- State Institute for Chemical and Veterinary Analysis of Food; R. Malisch, J. Haedrich, A. Kotz.
- Spanish Food Safety and Nutrition Agency of the Spanish Ministry of Health, Social Policy and Equality, Dr Ana Gago-Martinez (email: agagom@mspsi.es).
- Istituto Superiore di Sanità (ISS, Rome, Italy): R. Giordano.
- Ministry of Economic Affairs (EZ-DAD); L. Huizinga.

Contents

Summary

This report describes the activities employed in 2012 by RIKILT as National Reference Laboratory (NRL) in the field of dioxins and polychlorinated biphenyls (PCBs), pesticides in animal products, mycotoxins, heavy metals, polycyclic aromatic hydrocarbons (PAHs), and marine toxins according to Regulation (EC) No 882/2004 and Council Directive 96/23/EC.

For each domain, the EURL-NRL network is described and interactions and meetings within the network are reported. In 2012 RIKILT attended EURL meetings for each domain.

In the context of independent quality assurance, method validation and method comparison, RIKILT participated in proficiency tests, organised by the EURLs and third parties, and in interlaboratory validation and comparison studies. The results are reported and discussed. In general, the scope and quantitative performance of the methods used by RIKILT were satisfactory for all domains. Corrective measures were taken if the performance was not satisfactory.

Within the EURL-NRL networks on dioxins and pesticides, working groups discussed the performance criteria for screening methods and proposed changes in the existing regulations. Adapted criteria were published in 2012. In addition the feasibility of changing product definitions for animal products in the MRL pesticide legislation were discussed. Relevant information was exchanged with the competent authorities.

There were meetings and communications with official laboratories (OLs) in the Netherlands. Technical information was exchanged, analytical data on reference standards and reference samples were compared and conflicting analytical results were investigated.

This report also summarises some of the scientific research supporting the NRL activities as well as other analytical activities within other projects that contribute to maintaining the expertise in the respective NRL-analyte domains.

New developments are:

- For organic and free range egg contamination with dioxins, dioxin-like PCBs and PCBs a protocol was established to handle incidents in order to speed up finding sources of contamination. The criteria included recent knowledge on new sources that were found in 2012, like building debris and roof coatings.
- For products of animal origin the product definition for residues of pesticides have been harmonised with those of residues of veterinary drugs. This is especially important for fat soluble residues of pesticides.
- A new project will start in 2013 at RIKILT concerning identification criteria for mycotoxins analysed by LC-MS/MS.
- Measuring mercury with ICPMS was shown to be possible if isotope mass 201 is used, the mass without interference of tungsten oxide.
- A method was developed for analysis of PAHs (EU 15 + 1) in plant matrices, suitable to use for instance in case of huge fires resulting in potential PAHs deposition on grass, fruit and vegetables.
- The FVO audit on marine toxins in 2012 in the Netherlands confirmed adequate RIKILT performance.

1 Introduction

Residues and contaminants in food and feed can affect human and animal health. For many residues (pesticides, veterinary drugs) and contaminants (e.g. dioxins, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heavy metals, mycotoxins, processing contaminants) in food and/or feed, legislation has been established in which maximum residue limits (MRLs) or maximum levels (MLs) have been set. MRLs are set for residues of chemicals that are used in agricultural production chains with a purpose, like plant protection products and veterinary drugs. MLs are set for chemicals that end up in food and feed without being used in agricultural production as such. According to the regulation on official control, Regulation (EC) No 882/2004, member states should enforce food and feed law and monitor and verify that relevant requirements thereof are fulfilled by all food and feed business operators along production chains. This includes verifying compliance of food and feed with MRLs/MLs. Therefore, national monitoring and control plans, and EU coordinated monitoring programs have been established. As part of these programs, official samples are taken at various stages in the food chain and analysed in laboratories. To ensure the quality and comparability of the data generated by laboratories involved in the official monitoring and surveillance, a hierarchically structured system of European Reference Laboratories (EURLs), National Reference Laboratories (NRLs) and Official Laboratories (OLs) has been established.

RIKILT is involved in the official monitoring of various residues and contaminants in food and feed and has been assigned as NRL for dioxins/PCBs, mycotoxins, heavy metals and PAHs and marine toxins in the context of Regulation (EC) No 882/2004, and is the NRL on certain pesticides and toxic elements in animal derived products in the context of Directive 96/23/EC.

In addition, RIKILT is also NRL in other analyte/product domains (residues of veterinary medicines, hormones and contaminants in food of animal origin related to Directive 96/23/EC, genetically modified organisms (GMOs), animal proteins in feeding stuffs, additives in feed, water content in poultry meat, milk and milk products). Activities in those fields are outside the scope of this report and described in separate annual reports.

The tasks and responsibilities of the NRLs laid down in Regulation (EC) No 882/2004 (article 33) include:

a. Collaborate with the European Reference Laboratory in their area of competence;
b. Coordinate, for their area of competence, the activities of official laboratories responsible for the analysis of samples;
c. Where appropriate, organise comparative tests between the official national laboratories and ensure an appropriate follow-up of such comparative testing;
d. Ensure the dissemination to the competent authority and official national laboratories of information that the European Reference Laboratory supplies;
e. Provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans.

At the same time there is the requirement for the NRL to have accreditation on the specific compounds and matrices: "Competent authorities may only designate laboratories that operate and are assessed and accredited in accordance with the following European Standards:

a. EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories";
b. EN ISO/IEC 17011 on "General requirements for accreditation bodies accrediting conformity assessment bodies".

Member States that have more than one NRL for a specific EURL, must ensure that these laboratories work closely together, so as to ensure efficient coordination between them, with other national laboratories and with the EURL. One of the tasks of the NRL is therefore to communicate with the

Competent Authority (CA), other NRLs (where applicable) and OLs and other NRLs on issues regarding dioxins, dioxin-like PCBs (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs), PAHs, heavy metals, pesticides, mycotoxins and marine toxins. Where required, there are regular meetings with the competent authorities (EZ, NVWA, VWS) and other partners on:

- Content of the monitoring programs;
- Trends of contamination in feed and food of animal origin;
- Analytical methods, including validation and quality control procedures;
- European legislation;
- Incidents.

2 Dioxins, dioxin-like and non-dioxin-like PCBs

This chapter describes the activities of the NRL for dioxins and polychlorinated biphenyls (PCBs) in feed and food according to Regulation (EC) No 882/2004 and related activities in support of the expertise of RIKILT in this area. Also addressed are activities arising from the NRL task assigned to RIKILT in the context of Directive 96/23/EC (group B3a substances/chlorinated compounds including PCBs), i.e. dioxins and PCBs in animal derived products.

2.1 EURL-NRL Network

Table 1
EURL-NRL network for dioxins and PCBs in feed and food.

EURL	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg Postfach 100462 D-79123 Freiburg, Germany http://www.crl-dioxin-freiburg.eu/dioxinspcbs.html
NRL	Feed/food Animal products 96/23 B3a 22 (organochlorine compounds* including PCBs) RIKILT – Institute of Food Safety Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands W.A. Traag/Dr ir. L.A.P. Hoogenboom wim.traag@wur.nl/ron.hoogenboom@wur.nl

* For organochlorine pesticides, see Chapter 3.

2.1.1 Participation in EURL workshops

In 2012, RIKILT participated in two official workshops organised by the EURL in Freiburg (Germany), one in Vienna May 2012 and one in Freiburg November 2012.

2.1.1.1 Workshop in Vienna

During the first workshop in 2012 in Vienna Frans Verstraete (DG SANCO) gave an update on regulatory issues as regards PCDD/Fs and PCBs in feed and food at EU level. Below an overview of the current legislation is given in which maximum levels for PCDD/Fs and the sum of PCDD/Fs and dl-PCBs are set using TEFs-2005 instead of TEFs-1998 :

- Food: Commission Regulation (EU) No 1259/2011 of 2 December 2011 (OJ L 320, 3.12.2011, p. 18) (amending Regulation (EC) No 1881/2006)
- Feed: Commission Regulation (EU) No 277/2012 of 28 March 2012 (OJ L 91, 29.3.2012, p. 91) (amending Directive 2002/32/EC)

Action levels for PCDD/Fs and dl-PCBs are also calculated using TEF-2005 instead of TEF-1998. For some food groups action levels are no longer established:

- Food: Commission Recommendation of 23 August 2011 (2011/516/EU) (OJ L218, 24.8.2011, p. 23)
- Feed: Commission Regulation (EU) No 277/2012 of 28 March 2012 (OJ L 91, 29.3.2012, p. 91) (amending Directive 2002/32/EC)

New legislation on criteria for confirmatory and screening methods of analysis (PCDD/Fs, dl-PCBs and ndl-PCBs) were established in:

- Food: Commission Regulation (EU) No 252/2012 of 21 March 2012 (OJ L 84, 23.3.2012, p. 1) replaces Regulation (EC) No 1883/2006

- Feed: Commission Regulation (EU) No 278/2012 of 28 March 2012 (OJ L 91, 29.3.2012, p. 8) (amending Regulation (EC) No 152/2009)

After the update on legislation for food and feed Frans Verstraete gave an update on the German dioxin contamination incident as well as its legal follow up: Regulation (EU) No 225/2012 of 15 March 2012. This Regulation amended annex 2 to Regulation (EC) No 183/2005 and is applicable from 16 September 2012. Main changes introduced by this Regulation are:

- Feed businesses processing crude vegetable oils, manufacturing products derived from oils of vegetable origin and blending fats have to be approved by the competent authority (and not only registered as was the case).
- Fats intended for feed and food will now have to be strictly segregated during their production and transport from fats intended for technical use. The labelling of the products must explicitly mention their intended use. This declaration shall not be subsequently altered by an operator at a later stage of the chain.
- All laboratories are obliged to directly notify the competent authorities of any non-compliant finding of dioxins and dl-PCBs.

An EU harmonised monitoring plan with mandatory testing for dioxins depending on the risk inherent to the products will be introduced. The monitoring is targeted to the risky products at the moment they enter the feed chain. The analysis has to be performed in accredited laboratories and in accordance with Commission Regulation (EC) No 152/2009 (as amended).

A number of issues related to PCDD/Fs and PCBs are still under discussion e.g.: expression of the maximum level in liver from terrestrial animals on product or on fat basis (currently on fat basis), maximum level in sheep liver (deer liver), maximum level in food for infants and young children, maximum level in brown meat of mitten crab, safeguard measure dioxins (PCP) in guar gum from India following FVO inspection (see below under Workshop in Freiburg for more details).

During the workshop the following topics were presented by RIKILT:

Dioxins in hardened fat (hardened palm fatty acid distillates, HPFAD). An overview of a dioxin contamination incident in hardened fat for use in feed for ruminants in Denmark, with raw material from the Netherlands (RASFF notification 2011.1122-add01), and the performed studies was given. During the hardening of the fat a dechlorination of OCDD (and OCDF) to lower chlorinated PCDD/Fs was observed. This resulted in more toxic congeners, meaning an increase in TEQ-levels, and in addition, to non-2,3,7,8-congeners. Therefore more attention on OCDD in starting materials should be paid in future. As the source of OCDD is still unknown, more research on the source is necessary.

Dioxins in organic eggs. In July 2011 an egg sample from a farm in the Netherlands exceeded the maximum level for the sum of PCDD/Fs and dl-PCBs. Egg samples, collected from that farm in 2004, had shown levels clearly below the legal limits. During the next weeks investigations were performed including the analyses of several egg samples from the two different stables as well as soil and sand samples (from inside and outside). In the meantime the hens were kept inside. Additionally 10 eggs from each stable were tested individually with CALUX. The indicative, BEQ-levels in the individual eggs showed a considerable variation (range from around 5 to 30 pg BEQ/g fat). High levels were found in samples of gutter and also in non-used roof plates. It is questionable whether these levels are high enough to cause the contamination of the soil, and building debris applied for foundation of a new stable seems to be the most likely source of contamination. The use of contaminated debris on farms should clearly be avoided.

In order to prevent incidents, a regular sampling of feed (each new batch) is required to exclude feed as source of contamination. Additionally a sampling of hens (fat) or, of the first eggs of new hens, is necessary to exclude previous contamination. Also the courtyard should be shown to be clean before it is used for laying hens. When dioxin/PCB levels in eggs are increased, hens should be kept inside, after cleaning the inside of the stable to remove manure and soil. New samples after one and two weeks should be analysed to make the decision on maintaining the hens. This recent cases of contamination showed, that the limit for ndl-PCBs seems in some cases more prohibitive than the limit

for the sum of PCDD/Fs and dl-PCBs. The work presented by RIKILT was quite similar to the findings of high levels in organic eggs in North Rhine-Westphalia by CVUAMEL and in Lower Saxonia by BfR, findings that were presented by Thorsten Bernsman of CVUAMEL.

Prior to the official workshop in Vienna the core working group on MS/MS technology had a half day meeting to finish the discussion on the applicability of GC-MS/MS. Modifications and amendments of the current analytical criteria for confirmatory methods for determination of PCDD/Fs and dl-PCBs in feed and food were proposed, discussed and established. The draft document with the proposed modifications will be further discussed by DG SANCO with the Member States.

2.1.1.2 Workshop in Freiburg

During the second workshop in 2012 in Freiburg Frans Verstraete presented an overview of the RASFF notifications related to PCDD/Fs and PCBs in food and feed. From October 2011 until November 2012 in total 33 RASFF notifications were given, 22 related to feed and 11 to food. A number of on-going discussions concerning PCDD/Fs and PCBs were clarified by Frans Verstraete such as :

- Maximum level in liver from terrestrial animals on product or on fat basis (currently on fat basis): As all aspects have been comprehensively discussed, a decision will be taken at the next Standing Committee on Food Chain and Animal Health (SCFCAH) meeting on "POPs in Food" on 19 December 2012.
- Maximum level in sheep liver (deer liver).
- Maximum levels in food for infants and young children: In a draft EFSA opinion on PCDD/Fs in food for infants and young children proposition are made to change MLs. As soon as this Opinion will be published the SCFCAH will discuss this Opinion at its next meeting and will decide if MLs are to be changed.
- Dioxins in mitten crab: high levels of dioxins and PCBs were shown to be present in brown meat of mitten crabs, whereas the white meat was compliant with current maximum levels that are only applicable to this white meat. To protect public health the maximum levels should be applicable for whole meat, thus white meat and brown meat together. An European approach will be discussed at the next SCFCAH meeting.
- Dioxins and PCBs in organic eggs / free range eggs: high levels of dioxins and/or PCBs in free range eggs are a problem in several member states. It seems therefore appropriate to raise awareness and to find a common approach to deal with the problem of dioxins and PCBs in organic/ free range eggs.
- Presence of dl-PCBs and ndl-PCBs in food and feed tend to be more of a problem nowadays than presence of dioxins (PCDD/Fs).
- A strengthening of the official enforcement of provisions regarding feed and food is intended. In some special cases huge efforts are needed to get the last box of a contaminated product off the market (for example: contaminated picking stones for pigeons were distributed to a huge number of companies). Guidelines for follow-up in case of non-compliance for specific cases will be established.

Also during this workshop a new incident related to free range eggs was presented by RIKILT:

At RIKILT several cases of elevated levels of PCDD/Fs but more often PCBs were observed in eggs of free-range farms between 2010 and 2012 in the Netherlands and Germany. This can partly be explained by the fact that the self-control of companies has been strengthened and analysis includes more often PCBs. Farmers should check eggs of every new couple of hens at the age of 25-30 weeks. If the levels of PCDD/Fs and dl-PCBs are close to maximum levels, regular re-checks are necessary. In one of the positive cases a new source of contamination was discovered. It was shown that hens were contaminated on the farm where they were initially reared. From this farm samples of feed, soil, chicken fat and liver were analysed by RIKILT. In addition asbestos roofing sheets from the farm were analysed. It was concluded that the contamination was caused by a contamination during the rearing period of the hens, and that the contamination in the rearing period was caused by the asbestos roofing sheets coated with PCBs. At RIKILT a strategy was developed on what to do after elevated levels in free range or organic eggs are found. Related to a new incident RIKILT was asked to clarify (to a client) a report of analysis from a private laboratory regarding a sample of chicken fat containing high levels of PCBs. It became clear that the private laboratory used GC-MS-MS to confirm the

analytical results (which is a sophisticated technique). Formats of reports of analysis are, however, not harmonized and a report of analysis is not always understandable for a client.

In December an additional meeting was organized in Freiburg with two agenda items. One dealt with an initiative to develop a tool for using congener patterns to identify the source of the contamination. It was decided that the EU-RL will develop a format for collecting specific patterns which will subsequently be shared on the common CIRCA website. A number of NRLs will discuss the best way to develop a tool to use the patterns for source identification.

The second topic dealt with a further clarification of the use of screening methods, more specifically bioassays. The revised Regulation suggests that the former classification of a qualitative and quantitative approach was kept, whereas it was clearly decided that the sole purpose of bioassays was the selection of samples that might exceed the MLs. This was confirmed at the meeting and the current Regulation will be amended in the future. It was also decided that reference materials for bioassays must be spiked materials since incurred materials may contain non-regulated dioxin-like compounds, eventually leading to an undercorrection for recovery and hence an underestimation of the level.

2.1.2 Exchange of data with EFSA

As agreed with the ministries of EZ and VWS, RIKILT supplied data concerning dioxin and DL-PCB levels in Dutch feed and food to the European commission (DG SANCO) and EFSA. These data are used for exposure assessment but also to evaluate the EU-policy and to establish new maximum levels. With the help of RIVM the acceptance of the Dutch data by EFSA was ensured in 2012.

2.2 Proficiency tests and comparative tests

In 2012, RIKILT participated in the proficiency test (PT) organised by the EURL (i.e. pork meat and fat, and eggs). Results are presented in annex I. During the two EURL/NRL workshops in respectively Vienna and Freiburg the result of the PTs were discussed.

The first PT was on pork meat and pork fat RIKILT scored well, the results for all congeners were for the instrumental methods within the limits of (- 2 <z <2).RIKILT examined the samples also using the CALUX assay resulting in Z-scores of 0.7 en 0.6 for the two matrices.

The second PT was related to eggs, the results for all congeners were for the instrumental methods within the limits of (- 2 <z <2). RIKILT examined the samples also using the CALUX assay. Unfortunately results were reported after the deadline and were therefore not taken into consideration. It was shown afterwards that the CALUX results were correct when compared to the quantitative GC-HRMS results. Only small differences were observed.

2.3 Scientific and technical activities

The analytical competence in the field of dioxin analysis of both feed and food was maintained by analysing samples taken in the context of national control programs. Both CALUX screening and confirmatory GC-HRMS methods were applied. Scientific and technical support was given to the competent authority for the implementation of coordinated control plans. Together with the EURL and the University of Orebro (Sweden) the applicability of APGC, ionization at atmospheric pressure, followed by tandem MS was extensively studied and discussed. The initial results of this EURL / RIKILT study were presented at the opening of a "Centre of Innovation" at the University of Orebro.

RIKILT was closely involved in several dioxin incidents especially those concerning eggs from free range chickens. Also in 2012, at the request of and in close cooperation with the NVWA, again a number farms, where the levels were above the action level and /or the maximum levels, have been visited. Based on the inspections and results of analyses in relevant samples, so far in each case the

source of contamination could be detected. Next to soil as a source, contaminated building brick and roof plates coated with PCBs could be identified as new sources (see above). This new information about all kinds of different and quite often unexpected new sources was presented during EURL meetings, the dioxin-conference and a meeting with the Landeswirtschaftskammer Niedersachsen and KAT in Hannover. The latter because in Germany there are also problems with elevated levels of dioxins/PCBs in organic/free range eggs. A recommendation was set up for organic farms, which will also be translated to Dutch because many of these farms export eggs to Germany and have to follow the KAT-guidelines.

In 2012 relatively high dioxin levels were found in the floodplains of the river IJssel, resulting in contamination of grass pellets derived from these plains. It is not yet clear whether such high levels in floodplains also have consequences for animals grazing in these floodplain meadows.

On request of the Portuguese NRL RIKILT analysed a number of samples of chicken fat. The results of the analyses were in full agreement with the results obtained by the Portuguese NRL (levels were exceeding the maximum level).

2.4 Official sample analysis

In 2012 approximately 375 samples of a wide variety of feed commodities were analysed using DR CALUX and GC-HRMS. In addition, approximately 376 products of animal origin were obtained in the frame of the National Plan 96/23 and were analysed using DR CALUX and GC-HRMS. Results in food products will be available via the RIKILT website www.wageningenUR.nl/rikilt. In addition samples of sea fish, eel and mitten crab were analysed within the fish monitoring project and an additional project was carried out to investigate the incident on the farm that raised laying hens. On request of the advisory board of theme 4 (feed) an additional project was granted to make an inventory on the intake of soil as an additional source of dioxins and PCBs. Within Themes 1 and 4, the monitoring results in food and feed were reviewed and examined for trends.

2.5 Plan for NRL activities 2013

EURL workshop: in May an EURL/NRL meeting is planned at the Italian NRLs in respectively Rome and Teramo. End of 2012 a workshop will be held in Freiburg.

In close cooperation with the EURL for dioxins, RIKILT will further investigate the usefulness of a new tandem mass spectrometric technique base on atmospheric ionisation (APGC). Experiments on a system installed by the manufacturer at the EURL in Freiburg will be performed. At RIKILT an APGC user meeting will be organised in co-operation with WATERS (manufacturer) and the EURL. Scientist from The Netherlands, Germany, Sweden, Spain, England and the United States of America will be invited. These activities will be carried out within the new Expertise maintenance project.

The outcome of RIKILT research will be presented at the scientific conference Dioxin 2013 (Ron Hoogenboom and Wim Traag), August 25-30, Daegu, Republic of Korea.

Proficiency tests: RIKILT will participate in the following tests:

- Dioxins, PCBs and PBDEs in food organised by Folkehelse Instituttet (NIPH, Norway).
- Dioxins and PCBs in fat for production of animal feed organised by the EURL in Freiburg (Germany), both DR CALUX and GC-HRMS.
- Dioxins and PCBs in another product (to be announced) organised by the EURL in Freiburg.
- Dioxins, PCBs, POPs, PFAS in fish, sediment organised by UNEP.

2.6 Publications and presentations

Publications

Traag, W.A., Lee, M. vd, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCBs, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

Presentations

Hoogenboom L. Dioxines in de voedselketen, nog steeds een reden tot zorg. Presentatie tijdens Food Event, Utrecht.

Hoogenboom, L. Dioxins and dl-PCBs in the food chain. Presentatie tijdens workshop bij RIKILT over problematiek palmvetten.

Hoogenboom L. en Traag W. Dioxines in eieren. Studiegroep pluimvee. Roosendaal.

Hoogenboom L. and Traag W. Recent incidents with dioxins and PCBs. Oral presentation at the EURL-workshop in Vienna.

Hoogenboom L. Quality criteria for dioxin analysis with bioanalytical methods. Presentation at additional workshop at EURL, Freiburg.

Hoogenboom L. and Traag W. Recent incidents with dioxins and PCBs. Oral presentation at the POPs expert meeting in Brussels.

Hoang Thi T, Hoogenboom L, Traag W. Dioxins in free range consumption eggs from Vietnam: levels and health risks. Oral presentation by Traag at Dioxin 2012, Cairns

Haedrich J., Hoogenboom L.A.P., Eppe G., Goeyens L., Elskens M., Malagocki P., Scippo M.L., Vanderperren H., Windal I., Kotz A., Denison M.S. and Malisch R. Principles of method validation and quality control: compliance with the new EU-criteria for bioanalytical screening of feed and food. Oral presentation at Dioxin 2012, Cairns

Hoogenboom L, Dam G van, Immerzeel J, Oegema G, Kraats C van der Traag W. Problems with dioxins and dioxin-like PCBs in free-range eggs. Oral presentation at Dioxin 2012, Cairns.

2.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version.

- Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
- Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues, 98/536/EC
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC
- Commission Recommendation of 23 August 2011 on the reduction of the presence of dioxins, furans and PCBs in feed and food, 2011/516/EU
- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
- Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed
- Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006, Official Journal of the European Union L 84, 1-22.
- Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

3 Pesticides

This chapter describes the activities of the NRL for pesticides in products of animal origin arising from the NRL task assigned to RIKILT in the context of Directive 96/23/EC (group B2c substances/ carbamates and pyrethroids; group B3a substances/organochlorine pesticides; group B3b substances/ organophosphorus pesticides). This chapter also summarises related activities that supports the expertise of RIKILT in the field of pesticide residue analysis.

3.1 EURL-NRL-OL Network

Table 2

EURL-NRL-OL network for Pesticide residues in food and feed (http://www.crl-pesticides.eu).

	Food of animal origin and commodities with high fat content	Cereals and feeding stuffs	Fruits and vegetables, incl. commodities with high water and high acid content	Single residue methods
EURL	EURL-AO Chemisches und Veterinärunter-suchungsamt (CVUA) Freiburg Postfach 100462 D-79123 Freiburg Germany [Head Ralf Lippold]	EURL-CF National Food Institute Department of Food Chemistry Danish Technical University Moerkhoej Bygade 19 DK-2860 Soeborg Denmark [Head: Mette Poulsen]	EURL-FV Laboratorio Agrario de la Generalitat Valenciana (LAGV) Grupo de Residuos de Plaguicidas de la Universidad de Almería (PRRG) LAGV: E-46100 Burjassot-Valencia PRRG: E-04120 Almería Spain [Head: Amadeo Fernandez-Alba]	EURL-SRM Chemisches und Veterinärunter-suchungsamt (CVUA) Stuttgart Postfach 1206 D-70702 Fellbach Germany [Head: Michelangelo Anastassiades]
NRL	Laboratory of the Netherlands Food and Consumer Product Safety authority, Akkermaalsbos 4, 6708 WB Wageningen, The Netherlands H. Van Rhijn (MSc) Rhijn@minlnv.nl RIKILT – Institute of Food Safety* Akkermaalsbos 2 6708 WB Wageningen The Netherlands Dr. J.G.J. Mol Hans.mol@wur.nl	Laboratory of the Netherlands Food and Consumer Product Safety Authority (NVWA), Akkermaalsbos 4, 6708 WB Wageningen, The Netherlands Dr. A. De Kok, andre.de.kok@vwa.nl Koka4@minlnv.nl		
OL		Feed RIKILT – Institute of Food Safety Akkermaalsbos 2 6708 WB Wageningen The Netherlands Dr. J.G..J. Mol Hans.mol@wur.nl	Not applicable	Not applicable

* Organs/tissues, animal products (Directive 96/23/EC, B2c, B3a/b) (carbamates/pyrethroids/OCPs, OPPs).

http://www.nrl.rikilt.wur.nl/UK/Residues/Pesticides/

For the field of pesticides, four sub-domains have been established in the EU and consequently also in the member states (see Table above). The laboratory of the Netherlands Food and Consumer Product Safety Authority (NVWA) is NRL for all four domains in the framework of Regulation (EC) No 882/2004. RIKILT is NRL in the framework of Directive 96/23/EC. The NRLs also perform the major part of the official analyses for monitoring and enforcement. RIKILT performs virtually all analyses for pesticide residues in products of animal origin (non-processed commodities). Besides, RIKILT acts as OL for pesticide residues in feed and feed ingredients.

3.1.1 Participation in EURL workshops

In 2012 an EURL workshop for 'Pesticides in products of animal origin' was held in Freiburg, Germany (9-10 October). 37 representatives from 25 EU member states and Norway were present. The following topics were presented and discussed:

- Feedback from the Commission which included a change of desk officer pesticides DG SANCO (Luis Martin Plaza was not yet replaced) and the results of the evaluation of the EURL by the Commission (assignment will be extended on an annual basis as long as the EURL performs adequately).
- Presentation of the set up and results of the proficiency test organised by the EURL in 2012 (cream of milk, approximately 29% fat). In general, the results of the participating laboratories were adequate and better than in previous years. The robust standard deviation (Qn) was between 20-35% and close to the target fit-for-purpose RSD of 25%.
- Presentations by the NRLs of Sweden, Finland, France, Italy on the methods used for analysis of the PT samples.
- Multi-annual Coordinated Control Program: since 2012 a substantial number of 'new' pesticides have been included in the monitoring program. The selection was mainly based on a German assessment of exposure of animals through feed (as grown/fed in Germany) and data from registration dossiers indicating potential transfer. The determination of these new pesticides will be on voluntary basis in 2013 and then gradually become mandatory.
- Presentations on methods for the 'new' pesticides. In contrast to the classical pesticides that are typically determined by GC-based methods, LC-MS/MS is the method of choice for most 'new' pesticides. The EURL presented a comparison of different extraction methods. RIKILT presented the possibilities of generic extraction methods combined with various instrumental methodologies (LC-MS/MS, LC-HRMS, flow injection-MS/MS). A method for the determination of quaternary ammonium compounds (formally used as pesticides, but currently as disinfection agents, DDAC, BAC) based on QuEChERS extraction and LC-MS/MS analysis was also presented.
- On-going discussion and progress regarding clarification of the footnotes from annex 1of Regulation (EC) No 396/2005 (Regulation (EU) No 600/2010). Together with five other NRLs and the EURL, a proposal was submitted for simplification of the footnotes. This proposal was discussed with EFSA, adjusted, passed on to the Commission and accepted. The commodity descriptions will be changed into the 'raw agricultural commodity', i.e. muscle (without fat), fat, liver, kidney, offal, cow's milk, chicken eggs. Derived products (meat, dairy products etc.) will no longer be mentioned as such. This is also more consistent with the animal products in Regulation (EU) No 37/2010 for residues of veterinary medicinal products. Note: in the meantime the changes have been embedded in legislation (Regulation (EU) No 212/2013, effective per 1st April 2013).
- The matrix for the next EU proficiency test (2013) was discussed, candidate matrices were poultry and liver. Due to expected issues with stability of pesticides in homogenised liver (as already presented by RIKILT for certain pesticides) poultry was selected. The EURL will perform stability studies for liver to see whether this matrix will be feasible for 2014.
- The working program of the EURLs for 2013 was presented.

3.1.2 NRL-OL network

One of the tasks of the NRL is to communicate with the CA, OLs and other NRLs on issues regarding the control of levels of pesticide residues. With NVWA, besides joint attendance to the EURL workshop and symposia, a number of ad-hoc meetings were held. The main purpose of these meetings was to coordinate NRL tasks, to discuss analytical matters in order to have a joint opinion on issues to be discussed in EURL meetings (e.g. quality control, analytical methods) and to exchange technical

information. Input was provided for to the working group on the National Plan Residue monitoring with respect to the analysis scope.

RIKILT is chairing the user group for proficiency testing on organochlorine pesticides and PCBs in fats and oils, organised by KDLL to improve the quality of laboratories involved in feed analysis in the Netherlands. Proficiency test reports were evaluated and recommendations were given to the organiser.

3.2 Proficiency tests and comparative tests

RIKILT participated in various proficiency tests from the EURL and FAPAS. Results are presented in annex 2. It was noted that the two organisers used different target RSDs for calculating the Z-score: EURL a fit-for-purpose RSD of 25%; FAPAS the modified Horwitz RSD. The assigned value was the robust mean (mostly based on results not-corrected for recovery) in all cases.

The EURL-AO provided spiked samples of liquid cream (EUPT-AO7, 2012). The fat content was 29%. Besides the classical lipophilic pesticides also more polar pesticides that may end up in products of animal origin through feed intake were included in the scope. Due to inconsistent and confusing interpretation of MRLs in relation to the matrix, part of the results had to be expressed on product basis and part on fat basis. Various methods were used, based on either extraction of fat or the product as such, and based on LC-MS/MS or GC-MS/MS. All 15 spiked pesticides were detected and adequately quantified (Z-scores within -2/+2).

Because a lack of proficiency tests on animal fat, raw milk and eggs, RIKILT analysed pesticides in vegetable oil, milk powder and fish samples from the FAPAS proficiency test scheme and from RIKILT. All pesticides present were found and acceptable Z-scores obtained except for two cases of pyrethroids in salmon tissue. A follow up was done but no cause could be found. The samples were re-analysed and adequate results were obtained.

3.3 Scientific and technical activities

Technical assistance to the CA was focussed on a proposal for 2013 for adjustment of the scope for monitoring in products of animal origin. Current situation is that samples for monitoring pesticide residues in products of animal origin are taken in the framework of Directive 96/23/EC, and hardly any in the framework of Regulation (EC) No 882/2004. Because of this, the scope of the methods has always been focussed on chlorinated compounds (organochlorine pesticides and indicator PCBs), organophosphorus pesticides, carbamates and pyrethroids. In the past six years, these pesticides have hardly ever been detected. From EFSA reviews, draft assessment reports, and registration dossiers on pesticides, it is clear that residues may occur in animal products through intake of feed. This is also partially reflected in MRLs set in Regulation (EC) No 396/2005. So far, these pesticides have not been included in national or EU monitoring programs but in the current EU Multi-annual Coordinated Control Program (Regulation (EU) No 788/2012) a number of these pesticides are embedded (for the moment on a voluntary basis). The pesticides included are essentially based on a German assessment of registration dossiers and pesticides registered for use in crops used for animal feed in Germany. In 2012 an inventory has been made for the Dutch situation, based on literature and residue data from feed analysis. It was concluded that there are data gaps (pesticide use in the Netherlands, residue data in feed) and that the information is insufficient to set a very dedicated Dutch scope. In parallel, in method development and validation projects, it was demonstrated that the majority of the pesticides, that might be relevant to determine in products of animal origin, can be included in existing methods which reduces the necessity of establishing a very dedicated scope for the Dutch situation. A recommendation has been made to extend the existing methods with approximately 100 pesticides to simultaneously fulfil monitoring requirements from both Directive 96/23/EC and Regulation (EC) No 882/2004. In this way, the monitoring is also pro-actively changed towards a more risk-based approach.

3.4 Official sample analysis

Products of animal origin were analysed according to the National Residue Monitoring Plan in the framework of Directive 96/23/EC. In 2012 this involved analysis of approximately 170 meat samples for carbamates (LC-MS/MS), 230 samples of fat and milk for pyrethroids (GC-MS/MS), 450 samples of fat (tissue, milk, egg) for organochlorine pesticides and indicator PCBs (GC-MS/MS) and 190 samples of milk and liver for organophosphorus pesticides (LC-MS/MS). The analysis of approximately 250 animal feed commodities as part of the surveillance programs in the framework of Directive 2002/32/EC and Regulation (EC) No 396/2005 is also mentioned here, since this is relevant with respect to exposure of animals to pesticides through this route.

3.5 Plan for NRL activities 2013

EURL workshop: RIKILT will participate in the EURL workshop pesticides. This will be a joint meeting organised by the four EURLs in the field of pesticides in food and feed, autumn 2013, Almeria, Spain. During this meeting the revision of the analytical quality control guideline (SANCO/12495/2011) in which RIKILT is involved, will be presented and discussed.

NRL/OL network: Several meetings with the OL will be held to exchange information regarding monitoring and analytical issues.

Proficiency tests: RIKILT will participate in the EURL proficiency test for pesticides in products of animal origin (poultry) and in FAPAS proficiency tests for pesticides in milk powder and animal fat.

3.6 Publications and presentations

Publications

Traag, W.A., Lee, M. vd, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCBs, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

Tania Portoles, Hans G.J. Mol, Juan V. Sancho, Felix Hernández, Anal Chem. 2012 Nov 20; 84(22): 9802-10. Advantages of atmospheric pressure chemical ionization in gas chromatography tandem mass spectrometry: pyrethroid insecticides as a case study.

Presentations

Hans Mol, '(LC)/MS/(MS)-based methods for determination of pesticides residues in products of animal origin', lecture at EURL-AO workshop, 10 October 2012, Freiburg, Germany.

H.G.J. Mol, R.C.J. van Dam, P. Zomer, 'Flow injection – MS for rapid screening of pesticides not amenable to multi-residue methods: potential and limitations', Lecture at EPRW (European Pesticide Residue Workshop), Vienna, Austria, 25-28 June 2012.

Dini P. Venema, Paul Zomer, Theo C. de Rijk, Hans G.J. Mol, "Multi-residue analysis of pesticides in products of animal origin by generic extraction and LC-MS/MS", poster presented at EPRW (European Pesticide Residue Workshop), Vienna, Austria, 25-28 June 2012.

Hans G.J. Mol, Paul Zomer, Maarten de Koning, "EU identification criteria for LC-single stage high resolution MS: Are they fit-for-purpose?", poster presented at EPRW (European Pesticide Residue Workshop), Vienna, Austria, 25-28 June 2012.

3.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version

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

- Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues, 98/536/EC
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC
- Commission Implementing Regulation (EU) No 1274/2011 of 7 December 2011 concerning a coordinated multiannual control programme of the Union for 2012, 2013 and 2014 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin
- Commission Implementing Regulation (EU) No 788/2012 of 31 August 2012 concerning a coordinated multiannual control programme of the Union for 2013, 2014 and 2015 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin
- Regulation (EC) No 396/2005 of the European parliament and of the council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC
- Commission Regulation (EU) No 600/2010 of 8 July 2010 amending annex 1to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards additions and modification of the examples of related varieties or other products to which the same MRL applies
- Commission Regulation (EU) No 212/2013 of 11 March 2013 replacing annex 1to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards additions and modifications with respect to the products covered by that annex
- Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin
- Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed
- Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)
- SANCO/12495/2011, Method validation and quality control procedures for pesticides residues analysis in food and feed.
  http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdfReferences

4 Mycotoxins

This chapter describes the activities of the NRL for mycotoxins in accordance to Regulation (EC) No 882/2004 and related activities in support of the expertise of RIKILT in this area. A separate EURL and NRL have been appointed in the context of Directive 96/23/EC (group B3d), which focuses on the determination of mycotoxins in products of animal origin.

4.1 EURL-NRL Network

Table 3
EURL-NRL-OL network for Mycotoxins in food and feed.

EURL	Regulation (EC) No 882/2004: Joint Research Centre of the European Commission Geel, Belgium [Head: Joerg Stroka] http://irmm.jrc.ec.europa.eu/EURLs/eurl_mycotoxins/Pages/index.aspx Directive 96/23/EC: RIKILT – institute of Food Safety, Wageningen, Netherlands [Head: Saskia Sterk] http://www.eurl.rikilt.wur.nl/UK/General+information/
NRL	RIKILT – Institute of Food Safety Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands Dr. J.G.J. Mol Hans.mol@wur.nl http://www.nrl.rikilt.wur.nl/UK/Natural+contaminants/Mycotoxins/

4.1.1 Participation in EURL workshops

The annual workshop was organised by the EURL in Geel, Belgium (26-27 April 2012). 39 delegates from National Reference Laboratories attended the meeting and a number of invited speakers including Frans Verstraete (DG SANCO) and Mark Sykes (FERA/FAPAS). The following topics were presented and discussed:

- News from the EURL: changes in staff, overview of activities (training events) by the EURL in 2011 and an overview of planned activities for 2012.
- Presentation of the set up and results of the EURL proficiency test (PT) organised in 2012 for aflatoxin B1 in cereal-based babyfood, maize, animal feed and a standards solution.
- Andreas Breidbach (EURL) presented the results of an interlaboratory validation of a method for the determination of DON, ZON, T2/HT2 by LC-MS/MS. Options for calibration (matrix-matched vs ^{13}C-labels) were discussed.
- Joerg Stroka gave an update on the status of standardization of methods (CEN, AOAC) and the foreseen developments. For mycotoxins several method-standardization projects are expected in the second CEN mandate for food and third CEN mandate for feed.
- Frans Verstraete gave an update on EU legislation for mycotoxins. For various not yet regulated mycotoxins, EFSA opinions are being prepared and there is a need for generation of occurrence data. For feed the procedures for sampling/homogenisation are being revised and will be harmonised with those for food commodities.
- A separate session was devoted to proficiency testing and follow-up. The topic was introduced by the EURL and a representative from FAPAS and then further discussed in NRL-subgroups.

At the request of DG SANCO, the EURL started an expert working group to establish a guidance document for performance criteria for screening methods, including details on how to perform validation. RIKILT is participating in this working group. A first meeting was held in Geel (31 October 2012).

In November, RIKILT assisted the EURL in a training event on multi-mycotoxin analysis based on LC-MS/MS for NRLs that just implemented this technology. The training was held in Geel.

4.1.2 NRL-OL network

One of the tasks of the NRL is to communicate with the CA and OLs on issues regarding the control of levels of mycotoxins. A meeting was organised in which RIKILT and the OL presented their analysis methods for mycotoxins in various matrices, and results from recent monitoring and research projects. Besides, ad-hoc meetings were held and various symposia and NEN/CEN meetings were jointly attended. The main purpose of these meetings was to discuss analytical, sampling and occurrence matters.

Within the responsibility of RIKILT as NRL for mycotoxins, there is only one OL, which participated in the EURL proficiency test with acceptable performance and in addition to that in FAPAS proficiency tests. Based on the results obtained, no additional sample submissions for quality control purposes were considered necessary.

4.2 Proficiency tests and comparative tests

In 2012, RIKILT participated in various proficiency tests from the EURL, FAPAS and Coda-Cerva. Results are presented in annex 3. It was noted that the two organisers used different approaches for setting the assigned value. Where FAPAS uses the consensus value of the participants (robust mean), the EURL uses a value determined by themselves based on exact-matching double isotope dilution mass spectrometry.

The EURL provided two cereal mixtures that were naturally contaminated with DON, ZON, T2 and HT2. For DON and ZON good results were obtained (Z-scores within ± 1). For T2 and HT2 no Z-scores were provided because the robust mean of the participating laboratories deviated significantly from the value assigned by the EURL and no cause could be found at the time of reporting (Z-scores of RIKILT based on the consensus values were within ± 1).

The FAPAS proficiency test included aflatoxins in cereals, aflatoxin M1 in milk powder, ochratoxin in feed, T2/HT2 in feed, fumonisins in maize. Good Z-scores were obtained in all cases. The proficiency test organised by Coda-Cerva was a sample prepared from several materials on which fungi were grown. In this way a sample ‘naturally’ contaminated with 17 toxins was obtained. At the time of this report, no results were available yet.

4.3 Scientific and technical activities

RIKILT participates in the EFSA working group *Fusarium* toxins. In 2012 five meetings were held. Emphasis was on the preparation of a scientific opinion on nivalenol. Furthermore, work on beauvericine was initiated. RIKILT’s contribution focuses on methods of analysis, occurrence, processing and legislation.

Two meetings took place with experts of the university of Gent (group Sarah de Saeger), Belgium to discuss the occurrence and relevance of conjugated (‘masked’) mycotoxins.

Triggered by questions from the EURL-NRL network and literature, attention was paid to the extraction efficiency of fumonisins in naturally contaminated maize. It is known that the solvent and pH used for extraction can strongly affect extraction efficiency and this was experimentally confirmed. The extraction procedure used by RIKILT involves a single extraction using acetonitrile/water/formic acid. With this extraction solvent fumonisins can be extracted although it is recognized that for certain matrices the extraction is not quantitative. For this reason for fumonisins a procedure involving standard addition (to the sample) is used. Based on satisfying results obtained in proficiency tests in

the past years, it was concluded that this procedure provides comparable results compared to other laboratories using more polar solvents or multiple extractions.

Another technical activity concerned identification criteria for mycotoxins analysed by LC-MS/MS. So far there are no specific guidance criteria for identification of mycotoxins in food/feed. Due to this, criteria from other residue/contaminant domains (e.g. veterinary drugs [Decision 2002/657/EC] or pesticides [SANCO/12495/2011]) are often used. Using existing data from routine analysis of feed, ion ratios of mycotoxins in spiked samples were checked against those criteria. In a number of cases, mycotoxins did not meet the identification criteria. This calls for a more detailed assessment of qualitative LC-MS/MS performance and establishment of fit-for-purpose guidelines. A project proposal for this has been prepared for RIKIL's research/validation program of 2013. The topic has also been suggested to the EURL for the 2013 workshop.

4.4 Official sample analysis

In 2012 approximately 550 laboratory samples of a wide variety of feed commodities were analysed (LC-MS/MS multi-method, 33 mycotoxins) within the National Plan on feed. In addition, approximately 50 samples of milk/milk products were analysed for aflatoxin M1 (IAC, HPLC-fluorescence).

4.5 Plan for NRL activities 2013

EURL workshop: RIKILT will participate in the EURL workshop mycotoxins, scheduled for June 2013 in Rome, Italy. RIKILT will give a lecture on identification criteria for mycotoxins analysed by LC-MS/MS. In addition, RIKILT will continue its contribution to working group 'guidelines for screening methods'. NRL/OL network: several meetings with the OL will be held to exchange information regarding monitoring and analytical issues.

Proficiency tests: RIKILT will participate in the EURL proficiency tests foreseen for 2013 (patuline in apple juice, aflatoxin B1/DON/fumonisins in cereals, aflatoxin B1 in coconut). In addition, RIKILT will participate in other relevant proficiency tests organised by FAPAS or other parties.

4.6 Publications and presentations

Publications

Traag, W.A., Lee, M. vd, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCBs, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

Nijs, M. de, Top, H.J. van den, Portier, L., Oegema, G., Kramer, E., Egmond, H.P. van, Hoogenboom, L.A.P. 2012. Digestability and absorption of deoxynivalenol-3-β-glucoside in in vitro models. World Mycotoxin Journal 5:319-324.

Shephard, G.S.; Berthiller, F.; Burdaspal, P.; Crews, C.; Jonker, M.A.; Krska, R.; MacDonald, S.; Malone, R.J.; Maragos, C.; Solfrizzo, M.; Egmond, H.P. van; Whitaker, T.B., Developments in mycotoxin analysis: an update for 2010-2011. World Mycotoxin Journal 1 (2012)5. p. 3 – 30.

EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific Opinion on Ergot alkaloids in food and feed. EFSA Journal 2012;10(7):2798. [158 pp.]. doi:10.2903/j.efsa.2012.2798. Available online: www.efsa.europa.eu/efsajournal.

Presentations

Nijs, M. de, Pereboom-de Fauw, D.P.K.H., Rijk, T.C., Mol, J.G.J, Phomopsin A in lupin flour and lupin containing food in the Netherlands, poster presented at the 7th World Mycotoxin Forum / IUPAC mycotoxins, 5-9 november 2012, Rotterdam, Netherlands

Peters, J., Chan, D., Mallwitz, F., Haasnoot, W. The use of flow-cytometry and planar array based formats for multiplexed immuno-detection of mycotoxins in cereals and beer. Lecture at the 34th Mycotoxin Workshop, 14 - 16 May 2012 in Braunschweig.

Peters J., Darren Thomas, Frank Mallwitz en Willem Haasnoot. Multiplexed mycotoxin direct immuno-magnetic assay using xMAP technology and phycoerythrin-mycotoxin conjugates. Planet xMAP 10-11 October, Monaco, France.

Peters J., Diana Chan, Ruud van Dam, Theo de Rijk, Ronald van Doorn, David Katerere, Franz Berthiller, Willem Haasnoot, Han Zuilhofen and Michel Nielen. Development of a multiplex mycotoxin immunoassay and its application to 815 global beer samples. poster presented at the 7th World Mycotoxin Forum / IUPAC mycotoxins, 5-9 november 2012, Rotterdam, Netherlands.

Nijs, M. de, Van den Top, H.J., Portier, L., Oegema, G., Kramer, E., Van Egmond, H.P., and Hoogenboom L.A.P. 2012. Digestibility and absorption of deoxynivalenol-3-ß-glucoside in in vitro models. Lecture at 'Biotoxins Knowledge network Mycotoxins and plant toxins: emerging threats and their impacts on feed and food safety', November 29, Belfast Ierland.

Ad Jekel, Theo de Rijk, Hans van Egmond, Analytical aspects of the monitoring of T-2 and HT-2 toxins in Dutch food by duplicate diet collection in the period 1976-2006. Poster presented at the 7th World Mycotoxin Forum / IUPAC mycotoxins, 5-9 november 2012, Rotterdam, Netherlands.

Sara Stead, Dominic Roberts, Antonietta Gledhill, Theo de Rijk and Christof Van Poucke. The Development of a Sensitive Multi-Residue UPLC-MS/MS Method for the Quantitative Determination of Mycotoxins in Animal Feedstuffs and Silage. Poster presented at the 7th World Mycotoxin Forum / IUPAC mycotoxins, 5-9 november 2012, Netherlands.

M. Hove, P. Mngqawa, S.H. Ngobeni, D.R. Katerere, T.C. de Rijk, Multi-Mycotoxin Analysis of Food Crop Samples from Rural Northern South Africa. Poster presented at the 7th World Mycotoxin Forum / IUPAC mycotoxins, 5-9 november 2012, Netherlands.

Egmond Hans van, Mycotoxins: a global overview, Lecture at Conffidence/MycoDay, Liege, Belgium, March 2012.

Egmond Hans van, Mycotoxinen, stille belagers uit verleden en heden, lecture (gastcollege) at CAH Dronten, Netherlands, March/October 2012.

Egmond Hans van, Risicoschatting en regelgeving van mycotoxinen, lecture (gastcollege) at CAH Dronten, Netherlands, March/October 2012.

Fels-Klerx, Ine van der, Effects of climate change on crop phenology and occurrence, Lecture at Fusarium Toxin Forum, February 2012, Brussels, Belgium.

Egmond Hans van, MycoRed WP8, Information, education, dissemination, lecture at MycoRed General Assembly, April 2012, Budapest, Hungary.

Egmond Hans van, Human and animal health effects, risk assessment and mycotoxin legislation, lecture at MycoRed training workshop, March 2012, Bari, Italy.

4.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version

- Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
- Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues, 98/536/EC
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC
- Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding, 2006/576/EC

- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
- Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed
- Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs
- Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed
- Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC)
- SANCO/12495/2011, Method validation and quality control procedures for pesticides residues analysis in food and feed.
  http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdf

5 Heavy metals and toxic elements

This chapter describes the activities of the NRL for heavy metals in food and feed according to EU Regulation (EC) No 882/2004 and Directive 96/23/EC, and related activities in support of the expertise of RIKILT in this area. The focus is on the determination of cadmium, arsenic, lead and mercury in wild fish, meat, milk, food of plant origin and animal feed.

5.1 EURL-NRL Network

Table 4
EURL-NRL network for heavy metals in feed and food.

EURL	Food of Animal Origin: Istituto Superiore di Sanità Rome, Italy e-mail: crl@iss.it website: http://www.iss.it/lcdr Feed and food: Joint Research Centre of the European Commission Geel, Belgium http://www.irmm.jrc.be/EURLs/EURL_heavy_metals/Pages/index.aspx
NRL	Feed and food RIKILT – Institute of Food Safety Akkermaalsbos 2, 6708 PD Wageningen, The Netherlands Dr. M.K. van der Lee Martijn.vanderLee@wur.nl

5.1.1 Participation in EURL workshops

In 2012 RIKILT participated in workshops of the EURL for heavy metals in feed and food (IRMM, Geel, Belgium) and the EURL for chemical elements in food from animal origin (ISS, Rome, Italy).

The EURL workshop organised by IRMM was held in Brussels on 20th September 2012. During this workshop several issues related to the EURL activities and heavy metals in food and feed were presented and discussed:

- M.B de la Calle gave a presentation on the EURL activities in 2011 and 2012, and the work program for 2013 and 2014.
- The method of analysis for aluminium in noodles (discussion started by started by Joakim Engman).
- The statistical analysis of interlaboratory comparison results based on the ISO 13528 document.
- The main issue of the meeting was a discussion on the performance of the NRLs. During this discussion, emphasis was on the analysis of mercury in feed in proficiency test IMEP 114 – "The determination of total cadmium, lead, arsenic mercury and tin in feed premix". The outcome of the IMEP 114 proficiency test showed aberrant results for mercury for NRLs using ICP-MS. However NRLs using the cold vapour technique to analyse mercury in IMEP 114 sample showed good results for this element. This unexpected problem in the PT test was foreseen by the EURL. NRLs having both analytical techniques available in their laboratory were asked to investigate this issue in more detail. RIKILT was one of the laboratories that participated in this investigation.

The workshop in Rome on the 5th of October 2012 was organised by the EURL ISS. Items presented and discussed were:

- The results of the proficiency test organised by the EURL and performance of the NRLs.
- Arsenic speciation. A presentation on arsenic speciation was given by J. Sloth from DTU in Denmark focusing on possible methods for speciation of arsenic in food.

- Inorganic nanoparticles in food. A presentation was given by Francesco Cubadda on the detection and risk assessment of engineered inorganic nanoparticles in food. The focus was on special techniques for analysis and toxicity of the inorganic nanoparticles.
- The statistical processing of the proficiency test results
- Rounding off analytical results in relation to the maximum levels in Regulation (EC) No 1881/2006.

The NRLs were requested to spread the information received during the EURL/NRL workshops to the official laboratories (OL) and, more in general, to all laboratories performing analyses of food. One of the tools proposed was the inclusion of the link to the EURL webpage in the NRLs webpages.

5.1.2 NRL-OL network

Since 2012 the heavy metal department of the OL is located within the RIKILT building and knowledge exchange is almost on daily routine basis. During 2012 several unofficial meetings with the OL were held during which information concerning methods for the determination of heavy metals in food and feed were discussed. Also the PT results (from FAPAS and EURL-IRMM) were evaluated. Samples and reference materials were interchanged between NRL and OL to check the quality of reported results.

Unfortunately in 2012 the OL did not participate in the EURL organised proficiency test, because the matrix was a premix feed sample (IMEP 114). Upon request of RIKILT, the OL analysed the IMEP 114 sample on the ICPMS for heavy metals and mercury to investigate the “mercury-problem” discussed in Brussels earlier that year (see 5.3).

5.2 Proficiency tests and comparative tests

In 2012 the EURL for heavy metals in feed and food (IRMM, Geel, Belgium) and the EURL for chemical elements in food from animal origin (ISS, Rome, Italy) organised proficiency tests for heavy metals and toxic elements in food and feed in the framework of Regulation (EC) No 882/2004 and Directive 96/23/EC. RIKILT participated in these EURL proficiency tests as well as in proficiency tests organised by other organisers like FAPAS, KDLL and CODA-CERVA.

The proficiency tests from EURL-ISS included three matrices: infant formula, freeze-dried meat and milk. IRMM focused on Premix feed matrix for PT IMEP-114. For FAPAS matrices like powdered rice, canned fish, infant cereal, milk powder, Chilli powder and dairy ration were analysed. The matrices in the proficiency tests of KDLL were fish meal, premix and sow feed. For CODA-CERVA spinach was analysed.

In total 44 Z-scores were obtained for different element-matrices combinations. The elements included cadmium, lead, arsenic (total and inorganic fraction), mercury, iron and copper. For arsenic the average Z-score for 9 matrices was -0.2 (individual Z-scores ranged from-2.4 to 1.4). For inorganic arsenic the concentration in rice powder was below the detection limit of the method and thus no Z-score was calculated. To analyse products with low levels of inorganic arsenic in future the method was slightly modified. For cadmium the average Z-score in 12 matrices was 0.1 (range from -1.4 to 1.3). For mercury (n = 8) the average score was 0.0 (range from -0.4 to 0.8). Lead was measured in 9 proficiency test-matrices which resulted in 8 Z-scores. The average of these scores was 0.1 (range from -0.3 to 2.4). Results are presented in annex 4.

Lead in fish meal (proficiency test organised by KDLL) resulted in a Z-score of 9.1. This result led to closer investigation of the tested material and of the method of analysis used. The assigned value for lead in this fish meal was just above the LOD of the method. The standard addition technique used by RIKILT to make sure that the appropriate value was measured, appeared to be the cause of the high Z-score. However when the lead content was determined by RIKILT using the external calibration method the Z-score was acceptable, about 1.5. KDLL has informed RIKILT that the participating laboratories (n = 9) mainly used the external calibration method for the quantification and that the

assigned value was based on their results. Future discussions should focus on what is the best way to determine the exact level.

Furthermore, for arsenic in meat (PT EURL-ISS) and lead in feed premix (PT EURL-IRMM) Z-scores were obtained of -2.4 respectively 2.4. Therefore the analysis of arsenic in meat was redone and this time a Z-score of -0.7 was obtained. The method of analysis was slightly adjusted. The analysis of lead in premix was also redone, the new result based on external calibration method gave a Z-score of -0.5. RIKILT informed the EURLs on the procedure of quantification used and on if and when standard addition to matrices was applied The rather high Z-scores were reported at the end of 2012. The follow-up action is not completed yet.

5.3 Scientific and technical activities

Aberrant results for mercury in feed premix (IMEP 114) were obtained in the proficiency test by NRLs using ICPMS. In collaboration with the OL, RIKILT started experiments to elucidate possible causes of the aberrant results. From the experiments it was concluded that measuring mercury in the IMEP 114 sample using the ICP-MS is possible if isotope mass 201 is used, the mass without interference of tungsten oxide. When isotope mass 201 is used, the results of the ICPMS are similar to the results obtained by cold vapour technique. This conclusion was communicated to the EURL and published in the EURL their proficiency test report to inform the whole EURL-NRL-OL network on this issue.

5.4 Official sample analysis

In 2012 approximately 700 samples of a wide variety of feed commodities were analysed as part of the National monitoring Plan on feed. Products of animal origin were analysed in the framework of the National monitoring Plan 96/23. This plan involved the analysis of approximately 600 food samples.

5.5 Plan for NRL activities 2013

EURL workshop: as NRL heavy metals in food and feed and food from animal origin RIKILT will participate in the workshops of the EURL IRMM and EURL ISS organised in 2013.

NRL/OL network: within the scope of the quality assurance program, RIKILT will send reference materials and “unknown” samples to the OL in 2013. Furthermore the results of the PTs organised by the EURL (IRMM) or FAPAS will be discussed with the OLs. Information from the EURL workshops shall be shared with the OL contact persons during a meeting or via an e-mail/letter.

Proficiency tests: In 2013 RIKILT will participate in the following proficiency tests:
- PT organised by the EURL IRMM for food and feed samples.
- PT organised by the EURL ISS for food samples from animal origin.
- PT organised by FAPAS.

5.6 Publications and presentations

Traag, W.A., Lee, M. vd, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCB’s, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

5.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version

- Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules
- Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues, 98/536/EC
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC
- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs
- Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed
- Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and polycyclic aromatic hydrocarbons in foodstuffs
- Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed

6 Polycyclic Aromatic Hydrocarbons

This chapter describes the activities of the NRL for polycyclic aromatic hydrocarbons in feed and food according to Regulation (EC) No 882/2004 and related activities in support of the expertise of RIKILT in this area.

6.1 EURL-NRL Network

Table 5

EURL-NRL network for polycyclic aromatic hydrocarbons in feed and food.

EURL	Joint Research Centre of the European Commission Geel, Belgium http://irmm.jrc.ec.europa.eu/EURLs/EURL_PAHs/Pages/index.aspx
NRL	Feed and food RIKILT – Institute of Food Safety Akkermaalsbos 2, 6708 PD Wageningen, The Netherlands Dr. M.K. van der Lee Martijn.vanderLee@wur.nl

6.1.1 Participation in EURL workshops

RIKILT participated in the 7th workshop of the EURL for Polycyclic Aromatic Hydrocarbons (PAHs). This meeting was organised in Geel at 25-26 April 2012. Delegates of 23 NRLs were welcomed at the meeting by Thomas Wenzl. During the meeting the following issues were discussed:

- Since September 2012 new legislation concerning PAHs was published amending Regulation (EC) No 1881/2006 and Regulation (EC) No 333/2007. New maximum levels are set for benzo[a]pyrene (BaP) and the sum of 4 target PAHs: (benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF) and chrysene (CHR).
- The outcomes of PT PAHs in St John's wort and olive oil were discussed as well as the outcome of the PAHs survey in chocolate and cacao butter.
- The work program of 2012-2013 was discussed, and the EURL-NRL network agreed on the programme for 2013.

The NRLs were requested to spread the information received during the EURL/NRL workshops to the OLs and, more in general, to all laboratories performing analysis of food. One of the tools proposed was the inclusion of the link to the EURL webpage in the NRLs webpages.

6.1.2 NRL-OL network

In 2012 the OL mentioned a problem with the PAH congener concentrations in a newly bought standard solution from Dr. Ehrenstorfer GmbH. The incorrectness was found during routine control of this new standard solution before use. The OL concluded that some PAH congener concentrations seemed to be incorrect and on their request, RIKILT analysed this PAH standard mixture from Dr. Ehrenstorfer GmbH. RIKILT contacted the EURL after the analysis of the standard and sent the standard solution to IRMM. Both RIKILT and EURL-IRMM analysed the PAH mixture solution extensively and concluded that the response values of this precise solution seemed to be different compared to other standard solutions with the same PAH congener concentrations on the label. Upon this result the EURL contacted the standards supplier to take adequate follow-up actions. The PAH mixture should be withdrawn from the market (see annex VII).

For quality control purposes oil- and fat samples were sent to the OL. In December 2012 the OL analysed PAHs and the results were reported to RIKILT. The OL was informed about their performance via a report (Report 2012/RIK0302). The OL was contacted to discuss their results on PAH analysis and to share new information from the EURL on e.g. EU legislation.

6.2 Proficiency tests and comparative tests

RIKILT participated as NRL in proficiency tests organised by EURL-IRMM. Four indicator-PAH were measured in chocolate and cocoa butter, and olive oil. The Z-scores in olive oil are not yet reported, the Z-scores of the four indicator PAHs in chocolate and cocoa butter were all between -0.5 and 0. Furthermore RIKILT participated in PTs organised by FAPAS, which focused on the analysis of the EU 15 + 1 PAH in olive oil and 4 indicator-PAH in palm oil. The Z-scores obtained by RIKILT were between-2 and 2, except for two PAH congeners in olive oil: dibenzo (a, e) pyrene (+2.1) and dibenzo (a, l) pyrene (+3.0). These aberrant results were caused by an unstable measurement system. The samples were reprocessed and the results of the reanalysis gave good Z-scores of 0.32 and 0.17 respectively.

Thirdly RIKILT participated in PTs organised by KDLL focusing on the EU 15 + 1 PAH in lard, rapeseed oil, corn and palm oil. Just one out of the 56 results gave an erroneous Z-score, benzo (j) fluoranthene in rapeseed oil (Z-score was 5.8). This component co-eluted with benzo (b), (j) and (k) fluoranthene and the influence of the co-eluted PAH compounds was not appropriately taken into account. The data processing procedure was adapted and included in the standard operating procedure. The 55 other Z-scores were nicely in-between -2 and 2. The results are tabulated in annex V.

6.3 Scientific and technical activities

In 2012 a method has been developed for analysis of EU 15+1 PAH in plant matrices (endive, cauliflower, kale, spinach) and feed matrix. The method is based on extraction of PAHs from the sample matrix followed by a clean-up using gel permeation chromatography (GPC), and analysis by comprehensive two-dimensional gas chromatography with full scan time-of-flight mass spectrometric detection (GC×GC–TOF-MS). Qualitative and quantitative performance of the GC × GC system was evaluated by analysing spiked extracts in feed and plant matrices. All PAH compounds could be identified by the software based on their mass spectra. At lower levels the hit rate decreased with the concentration. System linearity was excellent in solvent and only slightly affected by matrix. Limits of quantification were 1–20 µg/kg for most PAH congeners. The overall method was validated for the 16 PAHs of interest at the 10 and 100 µg/kg level. The average trueness was 102% ranging from 75% to 130%.

6.4 Official sample analysis

RIKILT does not receive official samples taken in the context of monitoring programs for analysis for PAHs other than milk. On request of COKZ RIKILT analyses twenty milk samples for presence of PAHs twice a year. RIKILT was also asked to re-analyse samples of food supplements (taken on the market) for the OL.

6.5 Plan for NRL activities 2013

EURL workshop: As NRL-PAHs in food and feed RIKILT will participate in the workshop of the EURL organised in 2013.

NRL-OL network: within the scope of the quality assurance program RIKILT will send reference materials and “unknown” samples to the OL in 2013. Furthermore the results on the proficiency tests

organised by the EURL-IRMM, FAPAS or others will be discussed. Information from the EURL workshops shall be shared with the OL contact persons during a meeting or via an e-mail/letter.
Proficiency tests: in 2013 RIKILT will participate in the following proficiency tests:

- PT organised by the EURL IRMM for food and feed samples
- PT organised by FAPAS
- PT organised by KDLL

6.6 Publications and presentations

Traag, W.A., Lee, M.K. van der, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCB's, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

6.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version

- Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
- Commission Decision of 3 September 1998 establishing the list of national reference laboratories for the detection of residues, 98/536/EC.
- Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC.
- Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
- Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.
- Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and polycyclic aromatic hydrocarbons in foodstuffs.
- Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed.

7 Marine toxins

This chapter describes the activities of the NRL for marine biotoxins in food according to Regulation (EC) No 882/2004 and Regulation (EC) No 854/2004 and related activities in support of the expertise of RIKILT in this area.

7.1 EURL-NRL Network

Table 6
EURL-NRL network for marine toxins in food.

EURL	Spanish Food Safety Agency of the Spanish Ministry of Health Vigo, Spain http://www.aesan.msc.es/en/CRLMB/web/home.shtml
NRL	RIKILT – Institute of Food Safety Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands Dr A. Gerssen Arjen.Gerssen@wur.nl

7.1.1 Participation in EURL workshops

During the EURL annual meeting held in October 2012 in Belgium (WIP-ISP, Brussels) results of various PT studies and other issues were discussed. Paolo Caricato of DG-SANCO attended this meeting to share the vision of the EU Commission on several topics discussed in a CODEX meeting (1-5 October, Bali, Indonesia). He clarified that the Commission continues to promote the elimination of the mouse bioassay as legal requirement in both EU as well as international legislation or guidelines. Furthermore, deletion or raise of legal limits for yessotoxins (YTX) and pectenotoxins (PTX) is being considered by DG-SANCO. These toxins are not included in CODEX standards as there was not enough toxicological relevance to include these toxins in these standards. Therefore, DG-SANCO suggested that discussions should be initiated within the EU member states with the competent authorities in order to raise and/or delete limits for these toxins in EU legislation. DG-SANCO also confirmed that the Commission does not want to start discussions on (mainly) lowering legal limits for other toxins groups. This because, according to Paolo Caricato, if the recommendations from EFSA to lower certain limits are adopted the shellfish industry would be decimated.

DG-SANCO was asked if for paralytic shellfish poisoning (PSP) toxins the analogues to be monitored could be stated in legislation. Current EU legislation requires that if a standard for an analogue is available, the analogue should be included in the analysis. But some of the analogues for which standards are available have low toxicity equivalency factors (TEFs) and do not contribute significantly to the total toxicity. The EURL indicated that oral toxicity studies with certain analogues are being carried out in New Zeeland and Canada at this moment. The outcome of these studies will give the EURL more scientific evidence to decide on which PSP analogue to include or omit.

The EURL also gave an update on the establishment of the EU harmonised LC-MS/MS lipophilic toxin protocol as an Official Method of Analysis (OMA) in the AOAC. The AOAC refused to proceed with this method as an OMA method because of issues related to ownership and copyright of the work done within the EURL. Therefore the EURL, supported by the Commission, will look into the possibility to organise another collaborative trial including also laboratories from outside Europe (such as Japan, US, Canada and New Zeeland). The purpose of this new study is to establish a standardized method that is accepted globally and that will improve international trade. Finally, work done on method development for ciguatoxin and palytoxin was presented by the EURL.

Results of the annual proficiency testing (PT) on the amnesic shellfish poisoning (ASP) toxin domoic acid (DA) in shellfish were discussed during the annual EURL-NRL meeting. There was not much discussion on the ASP group as most laboratories had satisfactory results. After this the results of the PT on PSP toxins in shellfish were discussed. Two laboratories, including RIKILT, performed poorly in this PT. Both laboratories corrected for recovery while the other good performing laboratories did not take into account recovery. In a previous study conducted by the EURL in 2010 it was shown that recoveries of the various SPE procedures for PSPs contribute significantly to the overall recovery and the final results. Therefore laboratories were advised to be aware of their recoveries and apply corrections where needed. These corrections were not applied by most participants however. Furthermore, it became clear that there is a deviation between the mouse bioassay (MBA) and the analytical methodologies. All Z-scores below 0 belonged to the MBA method and those above 0 to the chemical methodologies. Therefore, it was concluded that separate Z-scores should be calculated for MBA and chemical methodologies. A revised report has been sent to the participants after the meeting. In the PT on lipophilic marine biotoxins most laboratories performed satisfactorily, clear improvements were obtained with the recently implemented LC-MS methods. There is a clear tendency towards the chemical test methodologies, out of a total of 38 results 21 were obtained by LC-MS (half of them using acidic mobile phase conditions and the other half alkaline mobile phase conditions), 2 by ELISA and 15 by MBA. This year no discrepancy between the MBA and chemical tests was observed. Only the ELISA missed the toxins in the first sample as the ELISAs used are designed for okadaic acid (OA) group toxins, and this first sample material contained only YTXs.

A general discussion was held on the target RSDs of the PT studies. Currently the target RSDs are determined using historical information, keeping in mind the difficulties for certain methodologies. Sometimes, for example for the PSPs, also data available from the collaborative studies are used. It was decided that a small working group should evaluate different options to calculate the target RSD. Members of this working group are NRL-IE (based on their Quasimeme experience), NRL-NL (based on the experience with collaborative studies) and some external experts. A meeting on RSDs is foreseen at the beginning of 2013.

Early May a seminar for working group members on new emerging toxins was organised in Vigo, Spain. During this meeting international experts were invited to give presentations on different "new emerging" toxin groups and on recent developments. Discussions were held on analytical methods, toxicology and legislation for cyclic imines (pinnatoxins and spirolides), palytoxins and ciguatoxins. RIKILT presented an updated LC-MS/MS method for lipophilic biotoxins including some cyclic imines (pinnatoxin-E, -F and G and 13-desmethyl spirolide C). With the updated method it is possible to analyse the regulated and some non-regulated toxins in shellfish extracts within 5 min. The most important conclusion of the meeting was that considering all recently published EFSA opinions, it is essential to prioritise future work on the basis of impact on health and economy. Ciguatoxins fit the best to these criteria and cyclic imines the slightest. It was decided that the working group on LC-MS in which RIKILT participates will contribute to the method development of several new emerging toxins. A WG meeting is foreseen early 2013.

7.1.2 NRL-OL network

In the beginning of 2012 results of a comparative test for lipophilic toxins (which was done in 2011) were discussed with the OL. Suggestions for improvements of the method for lipophilic marine biotoxins have been made. It was indicated by the OL that they would not perform marine biotoxin analyses in 2012. Therefore, no other comparative tests were organised between RIKILT and the OL. A shellfish sample that caused complaints to a consumer was investigated by RIKILT. The sample did not contain detectable levels of marine biotoxins.

7.2 Proficiency tests and comparative tests

In 2012, RIKILT participated in three PTs organised by the EURL. Results of the PTs were discussed during the annual EURL-NRL meeting (see above). For ASP toxins (DA) the outcome of the PT was partly satisfactory for the materials tested. RIKILT analysed the samples with both an LC-MS method

which is also applied in the routine control program and an LC-UV method which is the official method according to EU legislation. Material EURLMB/12/A/01 showed good Z-scores with both methods, thus Z-scores were between -2 and +2 (see annex VI for the complete summary of Z-scores). For the second material EURLMB/12/A/02, clam (*Ruditapes philippinarum*), with both methods an underestimation of the concentration occurred with Z-scores of -2.04 and -2.46 for respectively the LC-MS method and LC-UV method. Cause of this poor performance is most likely the extraction of DA from the matrix as the other material was analysed within the same series and there the Z-scores were satisfactorily. Therefore, it was decided if the following PT contains clam material, standard addition is used to quantify the amount of DA present.

For PSP toxins RIKILT participated with the Lawrence HPLC-FLD method. RIKILT performed poorly with Z-scores of 5.5 and 5.9. The blank material that was also provided was identified as blank. The poor performance is in our opinion caused by the different procedure followed by RIKILT for the construction of calibration curves. The RIKILT procedure is based on using matrix matched standards. Blank shellfish (mussels) are spiked at different toxin levels and these samples are used to construct a calibration curve. The spiked materials follow the same procedures as a regular sample, so extraction and the various solid phase extraction (SPE) steps are the same. As mentioned before in a recovery study conducted by the EURL in 2010 it was concluded that the SPE recoveries significantly contribute to the final result. Therefore, laboratories are advised to correct for their recovery. In the PT only two laboratories applied recovery correction and both laboratories were performing poorly compared to the other laboratories that did not take into account the recovery losses. Therefore, as follow-up study the samples were again analysed but this time quantified against standards in solution. The Z-scores of this study were good and within the -2 and +2 limits. It seems that recovery correction has, as expected, a major impact on the results and Z-scores. Therefore, in the next PTs for PSPs, results with and without recovery correction will be submitted by RIKILT. When evaluating the results of the PSP PT it became clear that there is a deviation between the MBA and the analytical methodologies. All Z-scores below 0 belonged to the MBA and above 0 to the chemical methodologies. Therefore, it was concluded that Z-scores should be calculated separately. A revised report has been sent to the participants.

In the PT on lipophilic marine biotoxins RIKILT performed good with Z-scores within the limits of -2 and +2 using the RIKILT LC-MS method. Only for one material containing OA group esters an error was made in the data interpretation resulting in a high Z-score for total OA after hydrolysis for this material. Further, it is good to notice that there is a clear tendency towards the use of LC-MS/MS methods instead of animal assays.

7.3 Scientific and technical activities

RIKILT participated in a working group meeting organised by the EURL on emerging toxins. Recent developments of LC-MS methods by RIKILT were presented. During this meeting it also became clear that due to issues at the EURL unfortunate they were not able to proceed with the AOAC to get the harmonised protocol for lipophilic toxins recognized as an Official Method of Analysis (OMA) within AOAC. Therefore, a discussion was held on which actions are necessary to get a LC-MS/MS method which is recognized by AOAC and ISO. It seems that a new study needs to be organised. In this new study additional toxins will probably added such as DA and some cyclic imines. In order to test if it is possible to incorporate DA into the lipophilic toxin method a preliminary study was organised. One of the PT materials for the lipophilic toxins (EURL-MB/ 12/L/1) contained DA. The request was to analyse the DA also with the traditional lipophilic methodology. Unfortunate only 6 laboratories fulfilled this request. For the acidic methodologies it seems possible to include DA. With the alkaline conditions which are used by RIKILT, it is not possible to get retention of DA on the analytical column. Further studies at the EURL are planned with respect to improvement of the extraction procedure as well as improvement of the chromatographic conditions.

An FVO audit team visited the Netherlands for bivalve shellfish. Within RIKILT the performance in the sanitary threshold program and the NRL tasks were evaluated. No suggestions were made by the audit team with respect towards improvement of certain tasks. RIKILT is still a member of the NEN task-

force mycotoxins and phycotoxins and member of the electronic working group of CODEX. In 2012 the CODEX e-WG proposed and revised several documents on performance criteria for screening and confirmatory methodologies. It is expected that this e-WG will be continued in 2013. The scope of the NEN taskforce mycotoxins and phycotoxins (part of CEN/TC 275/WG 5) was changed. Phycotoxins were omitted from the taskforce and phytotoxins were added. Therefore, Germany suggested a new CEN working group (CEN/TC 275/WG 14) on phycotoxins. If this suggestion is accepted, RIKILT will be put forward as representative of the Netherlands.

7.3.1 Incidents

In the course of 2012 two incidents occurred with marine biotoxins. In August there was an episode of *Alexandrium Ostenfeldii* in a recreational brackish water lake upstream of the shellfish production areas in the Eastern Scheldt. The water contained high levels of these toxic algae resulting in the presence of PSPs and cyclic imines (spirolides). The detection of these algae was triggered by the death of a dog after swimming in this lake. RIKILT analysed the stomach content of the dog and water samples and found the toxin profile specific for *Alexandrium Ostenfeldii*. RIKILT advised the product board of fisheries (PVis) on additional sampling of the shellfish in the production areas adjacent to the recreational lake. Shellfish analysis did not show the presence PSPs and cyclic imines in concentrations that could give rise to concern. The monitoring of additional samples continued till the algae problem in the lake was solved. A major concern was that possible rainfall would cause overflow of the lake into the shellfish production waters. Therefore, the local government together with the University of Amsterdam tried to reduce the number of toxic algae. This was done by addition of hydrogen peroxide to the water which caused death of the algae present. RIKILT investigated the effect of the treatment by measuring the levels of toxins in the water at certain time intervals after the treatment. After several treatments the procedure was considered as successful as the algae and toxin levels diminished.

A second incident occurred late October. In Belgium 112 people in a nursing home turned ill after consuming shellfish originating from a Dutch supplier. Symptoms suggested the presence of either diarrheic shellfish poisoning toxins (DSPs) or azaspiracids (AZAs). After research by the Belgium NRL the latter was the case. The shellfish could be traced back to import from Ireland (Castlemain Harbour). The imported shellfish were also released in five production areas in the Eastern Scheldt. Shellfish from these five areas were thus suspected to contain these toxins. The first analysis performed by RIKILT showed the presence of AZAs in various concentrations in shellfish from two different plots. Re-analysis by opening other shellfish from the same sample bag and performing additional analysis showed a high degree of heterogeneity between the shellfish samples, also confirmed by analysis of individual mussels. A relative large number of individual mussels contained levels far below the regulatory limit of 160 µg AZA1-eq/kg but a few mussels showed extremely high levels up to 2000 µg AZA1-eq/kg. NVWA asked RIKILT to give a scientific advice on this issue. In the advice it was concluded that for production areas suspected to contain the Irish shellfish, it could not be guaranteed that a negative analysis result implies that the shellfish from that specific plot are all below the regulatory limit. One parcel contained, according to the information present, mainly Irish shellfish while the other parcels contained mixtures of different shellfish originating from different production areas. The parcel containing predominantly Irish shellfish was designated as reference parcel. Regarding the reported half-life of AZA in shellfish, several months would be required to obtain levels that would ensure consumer safety. It was assumed that when the levels of AZAs in shellfish on this parcel were below the regulatory limit, the shellfish on the other parcels would also be below the regulatory limit. Due to the high level of heterogeneity a sampling strategy was suggested for sampling the reference parcel and to get good figures on the toxin levels present. In total 13 samples of 4 kg each (1 kg shellfish meat) were sampled and analysed. From the results it was clear that there is a large variation in the results (+/- 50%) and that the levels were above the regulatory limit. In the beginning of 2013 more data will be collected and statistical analysis will be performed. These outcomes can also be used for discussions on sampling strategies and how to deal with the sampling uncertainty in decision making.

7.4 Official sample analysis

RIKILT analysed on request of RIVM shellfish as part of the official control on areas where shellfish are grown and harvested. Furthermore, samples were analysed on request of Cyprus. Also on the request of the NVWA several shellfish samples have been analysed, a so-called complaint sample and suspected samples from the Eastern Scheldt in two incidents were analysed. In total 343 samples were analysed for ASP, 420 for PSP and 488 for lipophilic marine biotoxins.

7.5 Plan for NRL activities 2013

RIKILT will participate in the PTs for ASP, PSP as well as the lipophilic marine biotoxins organised by the EURL. RIKILT continues with the participation in the LC-MS working group on new emerging toxins and will participate in the new working g roup evaluating the target RSD. Furthermore, if needed assistance will be given to the OL as well as other NRLs in supporting the implementation or development of methods for the various marine biotoxins. Also continuation in CEN and CODEX activities will be of importance.

7.6 Publications and presentations

Presentations

Gerssen A. Improvement of the LC-MS/MS method for lipophilic marine toxins including pinnatoxins, Working group meeting on emerging toxins, 19-20 May, Vigo, Spain.

Gerssen A. Lipophilic marine biotoxins current and future perspectives, keynote lecture on the Quasimeme workshop, 14-15 June, Galway, Ireland

Current Developments In the Mass Spectrometric Detection for Lipophilic Marine Biotoxins, Harmfull algae bloom conference, 29 October – 2 November , Changwon, South Korea

The determination of hydrophilic toxins in shellfish, WMF meets IUPAC, 5-8 November, Rotterdam, The Netherlands.

Publications

Traag, W.A., Lee, M. vd, Mol, J., Gerssen, A., Leeuwen, S.P.J. van and Hoogenboom, L.A.P. (2012) Annual report National Reference Laboratories; Dioxins and PCBs, polycyclic aromatic hydrocarbons, heavy metals, mycotoxins, marine toxins and pesticides in animal derived products. RIKILT-rapport 2012.512.

7.7 References[1]

[1] All legal acts quoted in this report refer, where applicable, to the latest amended version

- Regulation (EC) No 882/2004 of the European parliament and of the council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.
- Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.
- Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.
- Alexander J, Benford D, Cockburn A, Cradevi J-P, Dogliotti E, Domenico AD, Fernandez-Cruz ML, Fink-Gremmels J, Fürst P, Galli C, Grandjean P, Gzyl J, Heinemeyer G, Johansson N, Mutti A,

Schlatter J, van Leeuwen R, van Peteghem C and Verger P. 2010. Scientific Opinion on marine biotoxins in shellfish – Emerging toxins: Ciguatoxin group. The EFSA Journal. 1627:1-38.

- Alexander J, Benford D, Cockburn A, Cradevi J-P, Dogliotti E, Domenico AD, Fernandez-Cruz ML, Fink-Gremmels J, Fürst P, Galli C, Grandjean P, Gzyl J, Heinemeyer G, Johansson N, Mutti A, Schlatter J, van Leeuwen R, van Peteghem C and Verger P. 2010. Scientific Opinion on marine biotoxins in shellfish – Cyclic imines (spirolides, gymnodimines, pinnatoxins and pteriatoxins). The EFSA Journal. 1628:1-39.

Annex 1 Overview of performance in proficiency tests on dioxins and PCBs in which RIKILT participated in 2012

Organisation	Matrix	Analyte	SOP nr.	Z-score
EU-RL	Pork Sausage	1,2,3,7,8-PeCDF	A0565	0.23
EU-RL	Pork Sausage	2,3,4,7,8-PeCDF	A0565	0.53
EU-RL	Pork Sausage	1,2,3,4,7,8-HxCDF	A0565	0.23
EU-RL	Pork Sausage	1,2,3,6,7,8-HxCDF	A0565	0.00
EU-RL	Pork Sausage	2,3,4,6,7,8-HxCDF	A0565	-0.04
EU-RL	Pork Sausage	1,2,3,7,8-PeCDD	A0565	0.55
EU-RL	Pork Sausage	1,2,3,4,7,8-HxCDD	A0565	0.29
EU-RL	Pork Sausage	1,2,3,6,7,8-HxCDD	A0565	0.28
EU-RL	Pork Sausage	1,2,3,4,6,7,8-HpCDD	A0565	0.37
EU-RL	Pork Sausage	OCDD	A0565	0.63
EU-RL	Pork Sausage	PCB 126	A0565	0.05
EU-RL	Pork Sausage	PCB 169	A0565	0.13
EU-RL	Pork Sausage	PCB 118	A0565	-0.21
EU-RL	Pork Sausage	PCB 105	A0565	-0.58
EU-RL	Pork Sausage	PCB 167	A0565	-0.34
EU-RL	Pork Sausage	PCB 156	A0565	-0.02
EU-RL	Pork Sausage	PCB 157	A0565	0.02
EU-RL	Pork Sausage	PCB 189	A0565	-0.08
EU-RL	Pork Sausage	PCB 153	A0565	-0.03
EU-RL	Pork Sausage	PCB 138	A0565	-0.35
EU-RL	Pork Sausage	PCB 180	A0565	0.16
EU-RL	Pork Sausage	WHO-PCDD/F-PCB-TEQ ub	A0565	0.21
EU-RL	Pork Sausage	WHO-PCDD/F-PCB-TEQ lb	A0565	0.82
EU-RL	Pork Sausage	WHO-PCDD/F-TEQ ub	A0565	0.75
EU-RL	Pork Sausage	WHO-PCDD/F-TEQ lb	A0565	1.98
EU-RL	Pork Sausage	WHO-PCB-TEQ ub	A0565	0.03
EU-RL	Pork Sausage	WHO-PCB-TEQ lb	A0565	0.18
EU-RL	Pork Sausage	Sum 6 Indicator PCBs ub	A0565	-0.44
EU-RL	Pork Sausage	Sum 6 Indicator PCBs lb	A0565	-0.35
EU-RL	Pork Sausage	% fat	A0565	0.23
EU-RL	Lard	1,2,3,7,8-PeCDF	A0565	-0.22
EU-RL	Lard	2,3,4,7,8-PeCDF	A0565	-0.07
EU-RL	Lard	1,2,3,4,7,8-HxCDF	A0565	0.07
EU-RL	Lard	1,2,3,6,7,8-HxCDF	A0565	-0.25
EU-RL	Lard	2,3,4,6,7,8-HxCDF	A0565	-0.09
EU-RL	Lard	1,2,3,7,8,9-HxCDF	A0565	0.20
EU-RL	Lard	1,2,3,4,6,7,8-HpCDF	A0565	0.67
EU-RL	Lard	1,2,3,4,7,8,9-HpCDF	A0565	0.34
EU-RL	Lard	OCDF	A0565	0.47
EU-RL	Lard	2,3,7,8-TCDD	A0565	0.92
EU-RL	Lard	1,2,3,7,8-PeCDD	A0565	0.42
EU-RL	Lard	1,2,3,4,7,8-HxCDD	A0565	0.23
EU-RL	Lard	1,2,3,6,7,8-HxCDD	A0565	-0.11
EU-RL	Lard	1,2,3,7,8,9-HxCDD	A0565	0.24
EU-RL	Lard	1,2,3,4,6,7,8-HpCDD	A0565	0.15
EU-RL	Lard	OCDD	A0565	0.05
EU-RL	Lard	PCB 81	A0565	-0.16
EU-RL	Lard	PCB 77	A0565	-0.24
EU-RL	Lard	PCB 126	A0565	-0.32
EU-RL	Lard	PCB 123	A0565	0.21
EU-RL	Lard	PCB 118	A0565	0.09
EU-RL	Lard	PCB 114	A0565	0.26
EU-RL	Lard	PCB 105	A0565	0.06
EU-RL	Lard	PCB 167	A0565	0.08
EU-RL	Lard	PCB 156	A0565	0.07
EU-RL	Lard	PCB 157	A0565	0.12
EU-RL	Lard	PCB 189	A0565	-0.08
EU-RL	Lard	PCB 28	A0565	-0.75
EU-RL	Lard	PCB 52	A0565	0.31

Organisation	Matrix	Analyte	SOP nr.	Z-score
EU-RL	Lard	PCB 101	A0565	0.27
EU-RL	Lard	PCB 153	A0565	-0.20
EU-RL	Lard	PCB 138	A0565	-0.01
EU-RL	Lard	PCB 180	A0565	-0.04
EU-RL	Lard	WHO-PCDD/F-PCB-TEQ ub	A0565	-0.04
EU-RL	Lard	WHO-PCDD/F-PCB-TEQ lb	A0565	0.09
EU-RL	Lard	WHO-PCDD/F-TEQ ub	A0565	0.51
EU-RL	Lard	WHO-PCDD/F-TEQ lb	A0565	0.51
EU-RL	Lard	WHO-PCB-TEQ ub	A0565	-0.57
EU-RL	Lard	WHO-PCB-TEQ lb	A0565	-0.48
EU-RL	Lard	Sum 6 Indicator PCBs ub	A0565	-0.03
EU-RL	Lard	Sum 6 Indicator PCBs lb	A0565	0.03
EU-RL	Egg - Whole	2,3,7,8-TCDD	A0565	2.60
EU-RL	Egg - Whole	1,2,3,7,8-PeCDD	A0565	0.30
EU-RL	Egg - Whole	1,2,3,4,7,8-HxCDD	A0565	0.40
EU-RL	Egg - Whole	1,2,3,6,7,8-HxCDD	A0565	0.50
EU-RL	Egg - Whole	1,2,3,7,8,9-HxCDD	A0565	0.40
EU-RL	Egg - Whole	1,2,3,4,6,7,8-HpCDD	A0565	0.60
EU-RL	Egg - Whole	1,2,3,4,6,7,8,9-OCDD	A0565	0.80
EU-RL	Egg - Whole	2,3,7,8-TCDF	A0565	0.30
EU-RL	Egg - Whole	1,2,3,7,8-PeCDF	A0565	0.90
EU-RL	Egg - Whole	2,3,4,7,8-PeCDF	A0565	0.30
EU-RL	Egg - Whole	1,2,3,4,7,8-HxCDF	A0565	0.60
EU-RL	Egg - Whole	1,2,3,6,7,8-HxCDF	A0565	0.30
EU-RL	Egg - Whole	2,3,4,6,7,8-HxCDF	A0565	0.70
EU-RL	Egg - Whole	1,2,3,7,8,9-HxCDF	A0565	0.80
EU-RL	Egg - Whole	1,2,3,4,6,7,8-HpCDF	A0565	1.40
EU-RL	Egg - Whole	1,2,3,4,7,8,9-HpCDF	A0565	1.00
EU-RL	Egg - Whole	1,2,3,4,6,7,8,9-OCDF	A0565	0.00
EU-RL	Egg - Whole	PCB 105	A0565	0.40
EU-RL	Egg - Whole	PCB 118	A0565	0.90
EU-RL	Egg - Whole	PCB 156	A0565	0.70
EU-RL	Egg - Whole	PCB 157	A0565	0.60
EU-RL	Egg - Whole	PCB 167	A0565	0.90
EU-RL	Egg - Whole	PCB 189	A0565	0.30
EU-RL	Egg - Whole	PCB 77	A0565	0.20
EU-RL	Egg - Whole	PCB 81	A0565	0.40
EU-RL	Egg - Whole	PCB 126	A0565	0.40
EU-RL	Egg - Whole	PCB 169	A0565	0.50
EU-RL	Egg - Whole	PCB 138	A0565	0.40
EU-RL	Egg - Whole	PCB 153	A0565	0.60
EU-RL	Egg - Whole	PCB 180	A0565	0.90
EU-RL	Egg - Whole	WHO-PCDD/F-PCB-TEQ ub	A0565	0.90
EU-RL	Egg - Whole	WHO-PCDD/F-PCB-TEQ lb	A0565	1.00
EU-RL	Egg - Whole	WHO-PCDD/F-TEQ ub	A0565	1.40
EU-RL	Egg - Whole	WHO-PCDD/F-TEQ lb	A0565	1.40
EU-RL	Egg - Whole	WHO-PCB-TEQ ub	A0565	0.70
EU-RL	Egg - Whole	WHO-PCB-TEQ lb	A0565	0.70
EU-RL	Egg - Whole	Sum 6 Indicator PCBs ub	A0565	0.80
EU-RL	Egg - Whole	Sum 6 Indicator PCBs lb	A0565	1.20
EU-RL	Egg - Whole	lipid content	A0565	-1.10
EU-RL	Egg - Yolk	2,3,7,8-TCDD	A0565	0.90
EU-RL	Egg - Yolk	1,2,3,7,8-PeCDD	A0565	0.40
EU-RL	Egg - Yolk	1,2,3,4,7,8-HxCDD	A0565	0.60
EU-RL	Egg - Yolk	1,2,3,6,7,8-HxCDD	A0565	0.20
EU-RL	Egg - Yolk	1,2,3,7,8,9-HxCDD	A0565	0.60
EU-RL	Egg - Yolk	1,2,3,4,6,7,8-HpCDD	A0565	0.50
EU-RL	Egg - Yolk	1,2,3,4,6,7,8,9-OCDD	A0565	0.80
EU-RL	Egg - Yolk	2,3,7,8-TCDF	A0565	0.20
EU-RL	Egg - Yolk	1,2,3,7,8-PeCDF	A0565	0.70
EU-RL	Egg - Yolk	2,3,4,7,8-PeCDF	A0565	0.30
EU-RL	Egg - Yolk	1,2,3,4,7,8-HxCDF	A0565	0.30
EU-RL	Egg - Yolk	1,2,3,6,7,8-HxCDF	A0565	0.00
EU-RL	Egg - Yolk	2,3,4,6,7,8-HxCDF	A0565	0.20
EU-RL	Egg - Yolk	1,2,3,7,8,9-HxCDF	A0565	0.70
EU-RL	Egg - Yolk	1,2,3,4,6,7,8-HpCDF	A0565	0.90
EU-RL	Egg - Yolk	1,2,3,4,7,8,9-HpCDF	A0565	0.70
EU-RL	Egg - Yolk	1,2,3,4,6,7,8,9-OCDF	A0565	-4.50
EU-RL	Egg - Yolk	PCB 105	A0565	0.90
EU-RL	Egg - Yolk	PCB 114	A0565	0.40
EU-RL	Egg - Yolk	PCB 118	A0565	0.90
EU-RL	Egg - Yolk	PCB 123	A0565	14.40

Organisation	Matrix	Analyte	SOP nr.	Z-score
EU-RL	Egg - Yolk	PCB 156	A0565	0.70
EU-RL	Egg - Yolk	PCB 157	A0565	0.80
EU-RL	Egg - Yolk	PCB 167	A0565	0.60
EU-RL	Egg - Yolk	PCB 189	A0565	0.50
EU-RL	Egg - Yolk	PCB 77	A0565	0.10
EU-RL	Egg - Yolk	PCB 81	A0565	0.50
EU-RL	Egg - Yolk	PCB 126	A0565	0.30
EU-RL	Egg - Yolk	PCB 28	A0565	0.30
EU-RL	Egg - Yolk	PCB 52	A0565	0.70
EU-RL	Egg - Yolk	PCB 101	A0565	0.70
EU-RL	Egg - Yolk	PCB 138	A0565	0.40
EU-RL	Egg - Yolk	PCB 153	A0565	0.40
EU-RL	Egg - Yolk	PCB 180	A0565	0.60
EU-RL	Egg - Yolk	WHO-PCDD/F-PCB-TEQ ub	A0565	0.70
EU-RL	Egg - Yolk	WHO-PCDD/F-PCB-TEQ lb	A0565	0.80
EU-RL	Egg - Yolk	WHO-PCDD/F-TEQ ub	A0565	0.70
EU-RL	Egg - Yolk	WHO-PCDD/F-TEQ lb	A0565	0.70
EU-RL	Egg - Yolk	WHO-PCB-TEQ ub	A0565	0.80
EU-RL	Egg - Yolk	WHO-PCB-TEQ lb	A0565	0.90
EU-RL	Egg - Yolk	Sum 6 Indicator PCBs ub	A0565	0.50
EU-RL	Egg - Yolk	Sum 6 Indicator PCBs lb	A0565	0.50
EU-RL	Egg - Yolk	lipid content	A0565	-0.60

Annex 2 Overview of performance in proficiency tests on pesticides in products of animal origin and high fat content in which RIKILT participated in 2012

Organizer	Matrix	Analyte	SOP	technique	Z-score
Fapas (580)	oily fish	chlorpyriphos (ethyl)	A1113	LC-MS/MS	-1.1
Fapas (580)	oily fish	chlorpyriphos-methyl	A1113	LC-MS/MS	0.2
Fapas (580)	oily fish	DDE, pp'	A0778	GC-MS/MS	0.7
Fapas (580)	oily fish	dieldrin	A0778	GC-MS/MS	0.3
Fapas (583)	milk powder	HCH gamma (lindane)	A0204	GC-MS/MS	-0.8
Fapas (583)	milk powder	diazinon	A0204	GC-MS/MS	*
Fapas (583)	milk powder	heptachlor	A0204	GC-MS/MS	0.3
Fapas (583)	milk powder	oxychlordane	A0204	GC-MS/MS	0.1
EUPT-AO7	milk cream (29% fat)	Bifenthrin	A0638	GC-MS/MS	-0.4
EUPT-AO7	milk cream (29% fat)	Chlordane cis-	A0204	GC-MS/MS	-0.1
EUPT-AO7	milk cream (29% fat)	Chlordane trans-	A0205	GC-MS/MS	-0.1
EUPT-AO7	milk cream (29% fat)	DDE p,p	A0206	GC-MS/MS	0.6
EUPT-AO7	milk cream (29% fat)	Diazinon	A1113	LC-MS/MS	-0.5
EUPT-AO7	milk cream (29% fat)	Deltamethrin	A0638	GC-MS/MS	0.4
EUPT-AO7	milk cream (29% fat)	Endosulfan sulfate	A0204	GC-MS/MS	1
EUPT-AO7	milk cream (29% fat)	Hexachlorobenzene (HCB)	A0204	GC-MS/MS	-0.3
EUPT-AO7	milk cream (29% fat)	HCH alpha-	A0204	GC-MS/MS	-0.2
EUPT-AO7	milk cream (29% fat)	HCH beta-	A0204	GC-MS/MS	-0.4
EUPT-AO7	milk cream (29% fat)	HCH gamma- (Lindane)	A0204	GC-MS/MS	-0.6
EUPT-AO7	milk cream (29% fat)	Parathion(-ethyl)	A0204	GC-MS/MS	0.9
EUPT-AO7	milk cream (29% fat)	Boscalid (F) (parent only)	A1113	LC-MS/MS	0
EUPT-AO7	milk cream (29% fat)	Tetraconazole (F)	A1113	LC-MS/MS	0.1
EUPT-AO7	milk cream (29% fat)	Thiacloprid (F)	A1113	LC-MS/MS	0.3
RIKILT	salmon muscle (B)	cypermethrin	A1113	LC-MS/MS	1.0
RIKILT	salmon muscle (C)	cypermethrin	A1113	LC-MS/MS	1.4
RIKILT	salmon muscle (D)	cypermethrin	A1113	LC-MS/MS	4.7 (1.0)**
RIKILT	salmon muscle (B)	deltamethrin	A1113	LC-MS/MS	2.6 (-0.6)**
RIKILT	salmon muscle (C)	deltamethrin	A1113	LC-MS/MS	2.0
* not provided					
** after re-analysis by both A0638 and A1113					

Annex 3 Overview of performance in proficiency tests on mycotoxins in food and feed in which RIKILT participated in 2012

Organizer	Matrix	Analyte	SOP	technique	Z-score
Fapas (4186)	animal feed cereal bas	aflatoxin B1	A0255	LC-MS/MS	0.4
Fapas (4186)	animal feed cereal bas	aflatoxin B2	A0255	LC-MS/MS	0.7
Fapas (4186)	animal feed cereal bas	aflatoxin G1	A0255	LC-MS/MS	0.0
Fapas (4186)	animal feed cereal bas	aflatoxin G2	A0255	LC-MS/MS	n.a.
Fapas (4186)	animal feed cereal bas	aflatoxin total	A0255	LC-MS/MS	n.a.
Fapas (17109)	animal feed	ochratoxin A	A0255	LC-MS/MS	n.a.
Fapas (2288)	animal feed	ZON	A0255	LC-MS/MS	0.5
Fapas (2289)	animal feed	DON	A0255	LC-MS/MS	0.9
Fapas (2282)	animal feed	T-2	A0255	LC-MS/MS	-0.2
Fapas (2282)	animal feed	HT-2	A0255	LC-MS/MS	-0.5
Fapas (4195)	milk powder	aflatoxin M1	A0255	LC-MS/MS	-0.8
Fapas (2287)	maize flour	fumonisin B1	A0255	LC-MS/MS	0
Fapas (2287)	maize flour	fumonisin B2	A0255	LC-MS/MS	0.5
Fapas (2287)	maize flour	fumonisins total (B1+B2)	A0255	LC-MS/MS	0.2
EURL Geel	cereal mixture (A)	DON	A0255	LC-MS/MS	-0.3
EURL Geel	cereal mixture (A)	ZON	A0255	LC-MS/MS	0.9
EURL Geel	cereal mixture (A)	T-2	A0255	LC-MS/MS	(0.5)
EURL Geel	cereal mixture (A)	HT-2	A0255	LC-MS/MS	(0.4)
EURL Geel	cereal mixture (B)	DON	A0255	LC-MS/MS	-0.4
EURL Geel	cereal mixture (B)	ZON	A0255	LC-MS/MS	0.3
EURL Geel	cereal mixture (B)	T-2	A0255	LC-MS/MS	(0.1)
EURL Geel	cereal mixture (B)	HT-2	A0255	LC-MS/MS	(0.0)
n.a. = not available					
between brackets: indicative Z-scores provided for information only					

Annex 4 Overview of performance in proficiency tests on heavy metals in which RIKILT participated in 2012

Organization	Matrix / Sample	Element	Method	z-score
CODA-CERVA	dried spinach	Arsenic - total	SOP A1057	*
CODA-CERVA	dried spinach	Cadmium	SOP A1057	0.2
CODA-CERVA	dried spinach	Mercury	SOP A1057	-0.1
CODA-CERVA	dried spinach	Lead	SOP A1057	-1.6
EURL-IRMM	Premix	Arsenic - total	SOP A1057	0.7
EURL-IRMM	Premix	Cadmium	SOP A1057	0.7
EURL-IRMM	Premix	Lead	SOP A1057	2.4
EURL-ISS	Infant Formula (powder)	Cadmium	SOP A1057	0.0
EURL-ISS	Infant Formula (powder)	Lead	SOP A1057	*
EURL-ISS	Meat - Freeze-Dried	Arsenic - total	SOP A1057	-2.4
EURL-ISS	Meat - Freeze-Dried	Cadmium	SOP A1057	1.3
EURL-ISS	Meat - Freeze-Dried	Lead	SOP A1057	-0.4
EURL-ISS	Milk - Liquid	Arsenic - total	SOP A1057	*
EURL-ISS	Milk - Liquid	Cadmium	SOP A1057	-0.8
EURL-ISS	Milk - Liquid	Lead	SOP A1057	-0.3
FAPAS	Rice, powdered	Arsenic - inorganic	SOP A1057	*
FAPAS	Rice, powdered	Arsenic - total	SOP A1057	0.0
FAPAS	Rice, powdered	Cadmium	SOP A1057	0.2
FAPAS	Fish - Canned	Arsenic - total	SOP A1057	0.0
FAPAS	Fish - Canned	Mercury	SOP A1057	0.8
FAPAS	Infant Cereal	Cadmium	SOP A1057	0.9
FAPAS	Infant Cereal	Mercury	SOP A1057	-0.3
FAPAS	Infant Cereal	Lead	SOP A1057	*
FAPAS	Fish - Canned	Arsenic - total	SOP A1057	0.1
FAPAS	Fish - Canned	Mercury	SOP A1057	-0.1
FAPAS	Milk powder	Arsenic - total	SOP A1057	*
FAPAS	Milk powder	Cadmium	SOP A1057	-0.1
FAPAS	Milk powder	Mercury	SOP A1057	-0.4
FAPAS	Milk powder	Lead	SOP A1057	0.5
FAPAS	Canned fish	Arsenic - total	SOP A1057	0.0
FAPAS	Canned fish	Cadmium	SOP A1057	-0.3
FAPAS	Canned fish	Mercury	SOP A1057	0.5
FAPAS	Chili Powder	Arsenic - total	SOP A1057	0.0
FAPAS	Chili Powder	Cadmium	SOP A1057	0.4
FAPAS	Chili Powder	Lead	SOP A1057	0.3
FAPAS	Dairy Ration	Iron	SOP A1057	0.7
FAPAS	Dairy Ration	Lead	SOP A1057	-0.3
FAPAS	Dairy Ration	Magnesium	SOP A1120	3.1
FAPAS	Dairy Ration	Manganese	SOP A1057	-1.1
FAPAS	Dairy Ration	Selenium	SOP A1057	-0.5
KDLL	Fish meal	Arsenic - total	SOP A1057	-1.2
KDLL	Fish meal	Cadmium	SOP A1057	0.1
KDLL	Fish meal	Mercury	SOP A1057	0.2
KDLL	Fish meal	Lead	SOP A1057	9.1
KDLL	Premix	Arsenic - total	SOP A1057	1.4
KDLL	Premix	Cadmium	SOP A1057	-1.4
KDLL	Premix	Mercury	SOP A1057	-0.4
KDLL	Premix	Lead	SOP A1057	-0.1
KDLL	Sow feed	Copper	SOP A1057	1.4
KDLL	Sow feed	Zinc	SOP A1057	1.2

*) Concentration <LOD; z-score is not calculated.

Annex 5 Overview of performance in proficiency tests on PAHs in which RIKILT participated in 2012

Organization	Matrix/Sample	Analyt	Method	z-score
EURL-IRMM	Cocoa Butter	benzo[a]anthracene	SOP A0834	0
EURL-IRMM	Cocoa Butter	benzo[a]pyrene	SOP A0834	-0.3
EURL-IRMM	Cocoa Butter	benzo[b]fluoranthene	SOP A0834	-0.5
EURL-IRMM	Cocoa Butter	PAH4 (sum)	SOP A0834	-0.5
EURL-IRMM	Chocolate	benzo[a]anthracene	SOP A0834	-0.1
EURL-IRMM	Chocolate	benzo[a]pyrene	SOP A0834	-0.3
EURL-IRMM	Chocolate	benzo[b]fluoranthene	SOP A0834	-0.5
EURL-IRMM	Chocolate	PAH4 (sum)	SOP A0834	-0.2
FAPAS	Oil - Olive	5-methylchrysene	SOP A0834	0.2
FAPAS	Oil - Olive	benzo[a]anthracene	SOP A0834	0.9
FAPAS	Oil - Olive	benzo[a]pyrene	SOP A0834	0.6
FAPAS	Oil - Olive	benzo[b]fluoranthene	SOP A0834	-0.3
FAPAS	Oil - Olive	benzo[c]fluorene	SOP A0834	-0.4
FAPAS	Oil - Olive	benzo[ghi]perylene	SOP A0834	-0.2
FAPAS	Oil - Olive	benzo[j]fluoranthene	SOP A0834	0.4
FAPAS	Oil - Olive	benzo[k]fluoranthene	SOP A0834	-0.1
FAPAS	Oil - Olive	cyclopenta[cd]pyrene	SOP A0834	0.3
FAPAS	Oil - Olive	dibenzo[a,e]pyrene	SOP A0834	2.1
FAPAS	Oil - Olive	dibenzo[a,h]anthracene	SOP A0834	0.3
FAPAS	Oil - Olive	dibenzo[a,h]pyrene	SOP A0834	1.7
FAPAS	Oil - Olive	dibenzo[a,i]pyrene	SOP A0834	0.8
FAPAS	Oil - Olive	dibenzo[a,l]pyrene	SOP A0834	3
FAPAS	Oil - Olive	indeno[1,2,3-cd]pyrene	SOP A0834	0.9
FAPAS	Oil - Palm	benzo[a]anthracene	SOP A0834	0.4
FAPAS	Oil - Palm	benzo[b]fluoranthene	SOP A0834	-0.1
FAPAS	Oil - Palm	benzo[a]pyrene	SOP A0834	0
FAPAS	Oil - Palm	indeno[1,2,3-cd]pyrene	SOP A0834	-0.1
FAPAS	Oil - Palm	benzo[ghi]perylene	SOP A0834	-0.2
FAPAS	Oil - Palm	PAH4 (sum)	SOP A0834	0.1
KDLL	Oil - Maize	5-methylchrysene	SOP A0834	0.4
KDLL	Oil - Maize	benzo[a]anthracene	SOP A0834	1.6
KDLL	Oil - Maize	benzo[a]pyrene	SOP A0834	1.9
KDLL	Oil - Maize	benzo[b]fluoranthene	SOP A0834	1.1
KDLL	Oil - Maize	benzo[c]fluorene	SOP A0834	*
KDLL	Oil - Maize	benzo[ghi]perylene	SOP A0834	1.1
KDLL	Oil - Maize	benzo[j]fluoranthene	SOP A0834	0.8
KDLL	Oil - Maize	benzo[k]fluoranthene	SOP A0834	1.4
KDLL	Oil - Maize	cyclopenta[cd]pyrene	SOP A0834	-0.6
KDLL	Oil - Maize	dibenzo[a,e]pyrene	SOP A0834	0.3
KDLL	Oil - Maize	dibenzo[a,h]anthracene	SOP A0834	0.8
KDLL	Oil - Maize	dibenzo[a,h]pyrene	SOP A0834	-0.8
KDLL	Oil - Maize	dibenzo[a,i]pyrene	SOP A0834	0.2
KDLL	Oil - Maize	dibenzo[a,l]pyrene	SOP A0834	-0.6
KDLL	Oil - Maize	indeno[1,2,3-cd]pyrene	SOP A0834	0.4
KDLL	Oil - Palm	5-methylchrysene	SOP A0834	0.2
KDLL	Oil - Palm	benzo[a]anthracene	SOP A0834	1.3
KDLL	Oil - Palm	benzo[a]pyrene	SOP A0834	1.5
KDLL	Oil - Palm	benzo[b]fluoranthene	SOP A0834	0.7
KDLL	Oil - Palm	benzo[c]fluorene	SOP A0834	*
KDLL	Oil - Palm	benzo[ghi]perylene	SOP A0834	1
KDLL	Oil - Palm	benzo[j]fluoranthene	SOP A0834	0.2
KDLL	Oil - Palm	benzo[k]fluoranthene	SOP A0834	1.7
KDLL	Oil - Palm	cyclopenta[cd]pyrene	SOP A0834	-1.2
KDLL	Oil - Palm	dibenzo[a,e]pyrene	SOP A0834	0
KDLL	Oil - Palm	dibenzo[a,h]anthracene	SOP A0834	1.1
KDLL	Oil - Palm	dibenzo[a,h]pyrene	SOP A0834	-0.6
KDLL	Oil - Palm	dibenzo[a,i]pyrene	SOP A0834	-0.6
KDLL	Oil - Palm	dibenzo[a,l]pyrene	SOP A0834	-0.3
KDLL	Oil - Palm	indeno[1,2,3-cd]pyrene	SOP A0834	1.4

Organization	Matrix / Sample	Analyt	Method	z-score
KDLL	Lard	5-methylchrysene	SOP A0834	1.6
KDLL	Lard	benzo[a]anthracene	SOP A0834	0.9
KDLL	Lard	benzo[a]pyrene	SOP A0834	0
KDLL	Lard	benzo[b]fluoranthene	SOP A0834	-0.2
KDLL	Lard	benzo[ghi]perylene	SOP A0834	0.6
KDLL	Lard	benzo[j]fluoranthene	SOP A0834	1.6
KDLL	Lard	benzo[k]fluoranthene	SOP A0834	0.7
KDLL	Lard	cyclopenta[cd]pyrene	SOP A0834	-0.6
KDLL	Lard	dibenzo[a,e]pyrene	SOP A0834	0.3
KDLL	Lard	dibenzo[a,h]anthracene	SOP A0834	0.7
KDLL	Lard	dibenzo[a,h]pyrene	SOP A0834	-0.6
KDLL	Lard	dibenzo[a,i]pyrene	SOP A0834	-0.6
KDLL	Lard	dibenzo[a,l]pyrene	SOP A0834	0.1
KDLL	Lard	indeno[1,2,3-cd]pyrene	SOP A0834	1.6
KDLL	Oil - Rapeseed	5-methylchrysene	SOP A0834	1.2
KDLL	Oil - Rapeseed	benzo[a]anthracene	SOP A0834	1.3
KDLL	Oil - Rapeseed	benzo[a]pyrene	SOP A0834	1
KDLL	Oil - Rapeseed	benzo[b]fluoranthene	SOP A0834	0
KDLL	Oil - Rapeseed	benzo[ghi]perylene	SOP A0834	0.9
KDLL	Oil - Rapeseed	benzo[j]fluoranthene	SOP A0834	5.8
KDLL	Oil - Rapeseed	benzo[k]fluoranthene	SOP A0834	1.5
KDLL	Oil - Rapeseed	cyclopenta[cd]pyrene	SOP A0834	-0.9
KDLL	Oil - Rapeseed	dibenzo[a,e]pyrene	SOP A0834	-0.2
KDLL	Oil - Rapeseed	dibenzo[a,h]anthracene	SOP A0834	0.9
KDLL	Oil - Rapeseed	dibenzo[a,h]pyrene	SOP A0834	-0.4
KDLL	Oil - Rapeseed	dibenzo[a,i]pyrene	SOP A0834	-0.5
KDLL	Oil - Rapeseed	dibenzo[a,l]pyrene	SOP A0834	0.1
KDLL	Oil - Rapeseed	indeno[1,2,3-cd]pyrene	SOP A0834	1.4

* No z-scores were reported.

Annex 6 Overview of performance in proficiency tests on marine biotoxins in bivalve molluscs in which RIKILT participated in 2012

Organizer	Matrix	Analyte	Method	Z-score	Remark
EURL-MB (12/A/1)	*Scallop Pecten Maximus*	ASP - DA	LC-MS (SOPA935)	-0.25	
EURL-MB (12/A/2)	*Clam Ruditapes philippinarum*	ASP - DA	LC-MS (SOPA935)	-2.04	
EURL-MB (12/A/1)	*Scallop Pecten Maximus*	ASP - DA	LC-UV (SOPA913)	-0.87	
EURL-MB (12/A/2)	*Clam Ruditapes philippinarum*	ASP - DA	LC-UV (SOPA913)	-2.46	
EURL-MB (12/P/1)	*Clam Venerupis pullastra*	PSP - total STX-eq	LC-FLD (SOPA1154)	5.50	
EURL-MB (12/P/2)	*Mytilus galloprovincialis*	PSP - total STX-eq	LC-FLD (SOPA1154)		Blank material
EURL-MB (12/P/3)	*Mytilus galloprovincialis and edulis*	PSP - total STX-eq	LC-FLD (SOPA1154)	5.90	
EURL-MB (12/P/1)	*Clam Venerupis pullastra*	PSP - total STX-eq	LC-FLD (SOPA1154)	1.20	Follow-up
EURL-MB (12/P/3)	*Mytilus galloprovincialis and edulis*	PSP - total STX-eq	LC-FLD (SOPA1154)	-1.03	Follow-up
EURL-MB (12/L/1)	*Mytilus spp*		LC-MS (SOPA1127)		Blank material
EURL-MB (12/L/2)	*Mytilus spp*	YTX	LC-MS (SOPA1127)	1.5	
EURL-MB (11/L/3)	*Mytilus spp*	Free OA-DTXs	LC-MS (SOPA1127)	-0.49	
EURLMB (11/L/3)	*Mytilus spp*	Total OA-DTXs	LC-MS (SOPA1127)	6.20	Error in interpretation
EURLMB (11/L/3)	*Mytilus spp*	AZAs	LC-MS (SOPA1127)	0.59	

Annex 7 Letter PAH

Ref. Ares(2012)1249329 - 23/10/2012

EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Reference Materials and Measurements (Geel)
European Union Reference Laboratory for Polycyclic Aromatic Hydrocarbons

EURL
European Union Reference Laboratory
Polycyclic Aromatic Hydrocarbons

Geel, 19.10.2012

Dear Colleagues,

The EU-RL PAHs was informed by the Dutch NRL about abnormalities reported by a Dutch official food control laboratory with lot 11214CY of Dr Ehrenstorfer Mix-183 in cyclohexane (LA20950183CY, expiry date 11/2014), containing the 16 EU priority PAHs at a nominal level of 10 mg/l.

Measurements at the EU-RL PAHs and at the Dutch NRL indicated that the concentrations of lot 11214CY were positively biased, meaning that the solution contains significantly higher amounts of PAHs than specified in the accompanying gravimetric certificate. Hence, applying the concentration values from the certificate for the calculation of standard concentrations would lead to the underestimation of the PAH content levels of the analysed food samples.

The EU-RL PAHs requested from Dr Ehrenstorfer GmbH clarification. Dr Ehrenstorfer GmbH confirmed the findings meanwhile and explained to the EU-RL PAHs that lot 11214CY will be retracted and a new lot will be produced.

If you were using lot 11214CY of Dr. Ehrenstorfer Mix-183 in the context of the official control of food, the EU-RL PAHs would like to urge you to check your calibrations against an independent reference, in order to prevent any wrong conclusions.

With best regards

Thomas Wenzl

(Operating manager of the EU-RL PAHs)

Retieseweg 111, B-2440 Geel - Belgium. Telephone: (32-14) 571 211. http://irmm.jrc.ec.europa.eu
Telephone: direct line (32-14) 571 320. Fax: (32-14) 571 783.

E-mail: jrc-irmm-crl-pah@ec.europa.eu

RIKILT Wageningen UR
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wageningenUR.nl/en/rikilt

RIKILT report 2013.007

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

The mission of Wageningen UR (University & Research centre) is 'To explore the potential of nature to improve the quality of life'. Within Wageningen UR, nine specialised research institutes of the DLO Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment. With approximately 30 locations, 6,000 members of staff and 9,000 students, Wageningen UR is one of the leading organisations in its domain worldwide. The integral approach to problems and the cooperation between the various disciplines are at the heart of the unique Wageningen Approach.

To explore the potential of nature to improve the quality of life

RIKILT Wageningen UR
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
www.wageningenUR.nl/en/rikilt

RIKILT report 2013.007

RIKILT Wageningen UR is part of the international knowledge organisation Wageningen University & Research centre. RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

The mission of Wageningen UR (University & Research centre) is 'To explore the potential of nature to improve the quality of life'. Within Wageningen UR, nine specialised research institutes of the DLO Foundation have joined forces with Wageningen University to help answer the most important questions in the domain of healthy food and living environment. With approximately 30 locations, 6,000 members of staff and 9,000 students, Wageningen UR is one of the leading organisations in its domain worldwide. The integral approach to problems and the cooperation between the various disciplines are at the heart of the unique Wageningen Approach.