Project numbers: 872.311.01, 872.312.01, 872.313.01, 872.314.01, 872.315.01 and 872.316.01
BAS number: WOT-02-438-I-028
Project title: National Reference Laboratory 882/2004

Project leaders: W.A. Traag, L.A.P. Hoogenboom, J.G..J. Mol

Report 2008.011 December 2008

National Reference Laboratory Dioxins, PCBs, Polycyclic Aromatic Hydrocarbons, Heavy metals, Pesticides and Mycotoxins in feed and animal products

Annual report 2007

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The research described in this report was funded by the Ministry of Agriculture, Nature and Food Quality, WOT Programme Foodsafety (theme Chemical Contaminants)

Distribution list:

- Ministry of Agriculture, Nature and Food Quality, Department of Food Quality and Animal Health (LNV-VD) ; Drs. E.R. Deckers, Dr. A. Meijering.
- Food and Consumer Product Safety Authority (VWA); Dr. R.M.C. Theelen, Dr. M.J.B. Mengelers, Drs. J.A. van Rhijn, Dr. A. de Kok, Mr. R. Herbes.
- Institute for Reference Materials and Measurements (JRC-IRMM, Geel, Belgium); Dr T. Wenzel.
- State Institute for Chemical and Veterinary Analysis of Food; Dr R. Malisch, Dr Johannes Haedrich, Dr A. Kotz.

Summary

This report describes the activities employed in 2007 by RIKILT as National Reference Laboratory (NRL) in the field of dioxins and polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heavy metals, pesticides and mycotoxins according to EU regulation 882/2004.

For each analyte domain, the CRL-NRL-OFL network is described and interactions and meetings within the network are reported. In 2007 RIKILT attended seven CRL meetings. In addition, there were several meetings and communications with other NRLs and laboratories. Technical information was exchanged. Analytical reference standards were compared and conflicting analytical results were investigated.

In the context of independent quality assurance, method validation and method comparison, RIKILT participated in proficiency tests, organised by the CRLs 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 analyte domains.

Also briefly summarised in this report is scientific research supporting the NRL activities as well as research which is embedded in other projects but contributes to maintaining the expertise in the respective NRL-analyte domains.

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1 Introduction

The European Commission is committed to protect both human and animal health and welfare, which can be affected by the presence of residues and contaminants in food and feed. For many residues (pesticides, veterinary drugs) and contaminants (e.g. dioxins, PCBs, PAHs, heavy metals, mycotoxins, processing contaminants) in food and/or feed, legislation has been established in which maximum limits (MRLs) have been set. European legislation commits the Member States to enforce compliance of food and feed with MRLs. Therefore, national monitoring and control plans, and EU coordinated monitoring programs have been established. As part of these programs, 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 Community Reference Laboratories (CRLs), National Reference Laboratories (NRLs) and Official Field Laboratories (OFLs) 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, PAHs, heavy metals, pesticides and mycotoxins in the context of EU regulation 882/2004. In addition, RIKILT is also NRL in other analyte/product domains (Residues of veterinary medicines and contaminants in food of animal origin related to EU directive 96/23, Genetically Modified Organisms, 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 are described in separate documents (RIKILT reports and can be found on the RIKILT website www.rikilt.wur.nl)

The tasks and responsibilities of the NRLs are laid down in EU regulation 882/2004 and include:
(a) collaborate with the Community 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 Community Reference Laboratory supplies;
(e) provide scientific and technical assistance to the competent authority for the implementation of coordinated control plans.

In the Netherlands, two NRLs have been appointed for all analyte domains included in this report.

Member States that have more than one National Reference Laboratory for a Community Reference Laboratory, must ensure that these laboratories work closely together, so as to ensure efficient coordination between them, with other national laboratories and with the Community Reference Laboratory.

One of the tasks of the NRL is to communicate with the Competent Authority, Official Field Laboratories and other NRLs on issues regarding the control of residues of dioxins and PCBs,

Polycyclic Aromatic Hydrocarbons, Heavy metals, Pesticides and Mycotoxins. Therefore, the communication and cooperation is restricted to the NRL food. There are regular meetings with the competent authorities (LNV, VWA , VWS) and other partners on:

- content of the monitoring programs.
- trends of contamination in feed and food of animal origin.
- analytical methods.

As for the determination of the contaminants of interest, there are no OFLs operational within the Netherlands.

2 Dioxins and PCBs

This chapter describes the activities of the NRL for dioxins and polychlorinated biphenyls (PCBs) in feed according to EU document 882/2004 [21] 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 [22] in the context of 96/23/EC [23] (group B3a substances/chlorinated compounds including PCBs), i.e. dioxins and PCBs in animal products.

2.1 CRL-NRL-OFL network

Table 1 CRL-NRL-OFL network for Dioxins and PCBs in feed and food

CRL[24]	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg Postfach 100462 D-79123 Freiburg Germany http://www.crl-dioxin-freiburg.eu/dioxinspcbs.html
NRL	**Feed** **Animal products 96/23 B3a** [22] (organochlorine compounds* including PCBs) RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands Dr.ir. L.A.P. Hoogenboom / W.A. Traag ron.hoogenboom@wur.nl / wim.traag@wur.nl **Food** Laboratory of the Food and Consumer Product Safety authority, De Stoven 22, 7206 AX Zutphen, The Netherlands H. van Rhijn (MSc) Hans.van.rhijn@vwa.nl
OFL	Not applicable

** For organochlorine pesticides, see Chapter 6.*

2.1.1 Participation in CRL workshops

In 2007, RIKILT participated in two workshops organised by the CRL in Freiburg (Germany). During the first of these workshops, criteria were discussed for the so-called reference method based on GC-HRMS.
The conclusion of the CRL/NRL network was that there is no need for adjusting the criteria as laid down in Commission regulation1883/2006 with exception of one proposed alteration (changing "reproducibility conditions RSD_R" to "within-lab reproducibility (RSD_{WR}).")

During the workshop in June the results of a proficiency test in food were extensively discussed. RIKILT participated in this test using both the confirmatory method (GC-HRMS) and a screening method (bioassay, CALUX). The results were within the acceptable limits established by the CRL.
In total only seven out of 100 laboratories (of which five NRLs) submitted results generated using the CALUX bioassay (for butter fat, chicken meat and salmon). Despite this limited data set and the fact that different types of extraction, clean-up, and cell-lines, had been used, an attempt was made to evaluate the data. The results thereof were presented during the workshop.
In most cases, the precision criteria for screening methods laid down in Commission regulation1883/2002 were not met. Only few Z-scores were within the range of +/- 2. This is probably caused by the use of different procedures both for the application and the interpretation of the CALUX assays. Also, this test only evaluated the quantitative approach and not the screening approach used by RIKILT. It was agreed that during a meeting in 2008 half a day would be dedicated to the use and interpretation of the CALUX bioassay.

The second workshop was held in November 2007 and was mainly focused on the results of a proficiency test with minerals as sample matrix and the guar gum incident (see below). The objective of the proficiency test for minerals was to check whether the previously developed method for sepiolite also could be used for other minerals such as 'Fuller's earth' and manganese oxide. The recommendations given for the extraction of sepiolite - using toluene + a substantial fraction of polar solvents - are also applicable for 'Fuller's Earth'. The highest recoveries[13] of the C-labelled standards when analysing manganese oxide were found when using toluene as extraction solvent. Digestion of the sample with HCl prior to extraction did not improve the recoveries. The additional use of polar solvents resulted in considerably lower recoveries. From the solvents tested, toluene was considered to be the most suitable extraction solvent for manganese oxide, but further improvement of the methods applied is still necessary.
The CRL provided comprehensive information on the appearance of contaminated guar gum on the European market in the summer of 2007. Guar gum was contaminated with pentachlorophenol with dioxins as co-contaminant. A summary was presented of the report of the FVO mission to India (DG (SANCO) 2007-7619-MR final). It was agreed that early 2008 a proficiency test with guar gum as sample matrix would be organised by the CRL.
Next to the above mentioned issues half a day was spent on analytical techniques. It was noted that in the case of the guar gom, contract laboratories systematically reported lower levels of dioxins compared to official laboratories. RIKILT was invited by the CRL to present a study on the comparison of automated extraction techniques.

2.1.2 NRL-OFL network

One of the tasks of the NRL is to communicate with the Competent Authority, Official Field Laboratories and other NRLs on issues regarding the control of levels of dioxin and PCB residues. Since for the determination of dioxin and PCB residues no OFLs are operational within the Netherlands, the communication and cooperation is restricted to the dioxin and PCB NRLs on food (VWA laboratory in Zutphen [AO]). At the request of the Dutch NRL dioxins in food (VWA Oost), a quality control sample program was designed to control the results of the screening test (DR CALUX) which is operated by VWA Oost.

Samples were prepared and sent to VWA Oost. The results of these QA samples will be statistically evaluated by RIKILT and the outcome will be discussed between RIKILT and VWA Oost.

As agreed with LNV and VWS, it was decided that RIKILT would supply data concerning dioxin and dl-PCB levels in Dutch produced feed and food to the European commission (DG SANCO). These data are used to evaluate the EU-policy and to establish new limits.

RIKILT chairs the Dutch working group dioxins ("Werkgroep dioxinen"). This is a nation-wide working group consisting of all relevant ministries and institutes dealing with all kinds of aspects of dioxins and persistent organic pollutants in food, feed and the environment. During a meeting in November 2007 the occurrence of high levels of dioxins in eel from Dutch rivers was discussed.

2.2 Proficiency tests and comparative tests

In 2007, RIKILT participated in three proficiency tests on dioxins and PCBs. Relevant data are provided in Appendix 1. One proficiency test was organised by the Norwegian Institute of Public Health (Folkehelse instituttet) in cooperation with the CRL. The test involved analysis of three different commodities (salmon, chicken meat, butter). In this case the determination of the fat content was an inherent part of the analysis and therefore results are included in the Appendix. The other two proficiency tests were organised by FAPAS and concerned vegetable oil and fish oil. For determination of dioxins and PCBs a confirmatory method based on GC-HRMS was used. In one proficiency test dedicated to determination of indicator PCBs, a GC-ECD method was used. The performance of RIKILT in the proficiency tests was good, all Z-scores obtained were within -2 and +2.

Early 2007 RIKILT reported a level of dioxins in a sample of sepiolite which exceeded the maximum residue limit by a factor of approximately eight. The producer of the batch sepiolite requested for an appeal sample which was submitted for analysis by a private laboratory accredited for dioxin analysis. This laboratory could not confirm the MRL violation.
Upon request of RIKILT, additional samples were taken by the competent authority to be analysed by RIKILT. All samples exceeded the MRL, confirming the earlier findings. To settle the dispute, RIKILT initiated a third sample to be taken which was sent to the CRL in Freiburg. The results of the analysis by the CRL confirmed the results reported by RIKILT.

2.3 Scientific and technical activities

Within the framework of the NRL task, upcoming new analytical methods have been studied. In order to increase the efficiency of sample preparation a large volume injector has been implemented in the GC-HRMS. The scope at the method for the determination of dioxins and PCBs was extended to flame-retardants. This study would be reported in 2008.

Accreditation and practical experience are important requirements for an NRL. In 2007 the accreditation for both the HRGC/HRMS and the DR CALUX was prolonged. A number of additional validation studies were started. The analytical competence in the field of dioxin analysis of both feed and food was

maintained through analysis of samples taken in the context of national control programs. Both CALUX screening and confirmatory GC-HRMS methods were used.

The relative potencies of the various dioxin and dl-PCB congeners in the DR CALUX assay were studied. This is important for investigations into the differences that might be obtained when using the DR CALUX bioassay and the confirmatory GC-HRMS method. It is also relevant for the identification of novel risks. A method was developed for the identification of novel agonists in food and successfully used to identify the most important natural Ah-receptor agonist in marmalade. The method has later been applied on grass samples that show a false-positive result in the DR CALUX assay.

2.4 Plan for NRL activities 2008

In 2008 RIKILT participates in the following Proficiency tests:

- Dioxins, PCBs and PBDEs in food organised by Folkehelsa (NIPH, Norway).
- Dioxins and pentachlorophenol in guar gum organised by CRL Freiburg and Stuttgart (Germany).
- PBDEs in cod liver oil organised by FAPAS (United Kingdom).
- Dioxins in oil organised by FAPAS (United Kingdom).

RIKILT supports the CRL Freiburg with their CALUX activities. The first meeting is held in March 2008 at RIKILT.

RIKILT reports the analytical data obtained during the last 4-5 years in the Netherlands to DG SANCO.

Participation in the workshops of the CRL and Dioxin 2008 in Birmingham.

Organisation of 1-2 meetings of the Working Group on Dioxins.

2.5 References

[21] 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, Official Journal of the European Union L 191, 28.5.2004, P 01-52.

[22] Commission decision No 2006/130/EC of 10 February 2006 amending Decision 98/536/EC establishing the list of National Reference laboratories for the detection of residues (notified under document number C(2006) 330).

[23] 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, Official Journal L 125 , 23/05/1996 P. 0010 – 0032.

[24] COMMISSION REGULATION(EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories.

2.6 Publications and presentations

Peer reviewed papers and reports:

Hoogenboom, L.A.P., Van Eijkeren, J.C.H., Zeilmaker, M.J., Mengelers, M.J.B., Herbes, R., Immerzeel, J., Traag, W.A. (2007) A novel source for dioxins present in recycled fat from gelatin production. Chemosphere 68, 814-823.

Leeuwen, S.P.J. van, Leonards, P.E.G., Traag, W.A., Hoogenboom, L.A.P., Boer, J. de (2007) Polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in fish from the Netherlands: concentrations, profiles and comparison with DR CALUX® bioassay results. Analytical and Bioanalytical Chemistry 389, 321-333.

Kijlstra, A., Traag, W.A. and Hoogenboom, L.A.P. (2007) Effect of flock size on dioxin levels in eggs from chickens kept outside. Poultry Science 86, 2042-2048.

2007-003 Report on Dioxins and dl-PCBs in eel from the large rivers in the Netherlands (in cooperation with IMARES).

Contributions at Conferences and Workshops:

Hoogenboom L.A.P., Kotterman M.J.J., Zeilmaker M.J., Hoek-van Nieuwenhuizen M., van der Lee M.K., Traag W.A. (2007) Dioxin and PCB levels in eel from large rivers in the Netherlands, the impact of the 2006 TEF values, and the risk for the consumer. Organohalogen Compounds 69: 122-125. Oral presentation at Dioxin 2007, Tokyo.

Heneweer M., Peijnenburg A.A.C.M., Poortman J.H., Baykus H., Hoogenboom L.A.P. (2007)TCDD exposure results in differential expression of α2U-Globulin in young male rats. Organohalogen Compounds 69: 1874-1877. Poster presentation at Dioxin 2007, Tokyo.

Liza Portier, Gerrit Bor and Ron Hoogenboom, Strategies for using the DR CALUX® assay in dioxin and dl-PCB analysis. Poster presentation at Recent Advances in Food Analysis, Prague 2007.

Ede, K. van, Antunes-Fernandez E., Li A., Mulder P. and Hoogenboom R. Bioassay directed analysis of Ah-receptor agonists in citrus fruit. Oral presentation at Recent Advances in Food Analysis, Prague November 2007.

Hoogenboom L.A.P. Dioxins in feed and food. Oral presentation at BDS workshop, October 2007, Amsterdam.

3 Polycyclic Aromatic Hydrocarbons

3.1 CRL-NRL-OFL network

Table 2 CRL-NRL-OFL network Polycyclic Aromatic Hydrocarbons

CRL[31]	The Joint Research Centre of the European Commission Geel, Belgium http://www.irmm.jrc.be/html/CRLs/crl_pah/index.htm
NRL	**Feed** RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands W. A. Traag (MSc) Wim.traag@wur.nl **Food** Laboratory of the Food and Consumer Product Safety Authority (VWA), Veldm. Montgomerylaan 500, 5623 LE Eindhoven, The Netherlands J. van der Wielen (MSc) Jacqueline.van.der.wielen@vwa.nl
OFL	Not applicable

3.1.1 Participation in CRL workshops

In February 2007 a workshop was held in Geel. The results of a proficiency test organised in 2006 were discussed. RIKILT was not able to participate in that proficiency test because at that time the Netherlands had not yet notified the CRL on the NRL assignment. The test involved the analysis of a standard solution. The outcome of this test was disappointing. Reported values deviated strongly. It was not really possible to indicate a clear error. The majority of the laboratories that had participated used different procedures (two analytical approaches, GC and HPLC, with varying conditions). Due to the many variables it was not clear which factor was responsible for the variability in the results.
During this workshop the method used by RIKILT (GPC in combination with GC-HRMS) was presented. Two other methods were presented in lectures by other NRLs.
During the plenary discussion it became clear that there is no single method that can detect all PAHs from the EU priority list (15 +1) at the relevant level in one run. In November 2007, RIKILT participated in the second proficiency test involving the analysis of 15+1 EU priority PAHs in edible oil and solvent solution. The results of this test are discussed in 2008.

3.1.2 NRL-OFL network

During several meetings, information concerning methods of analysis in a variety of matrices has been discussed with VWA Eindhoven. Methods and results with respect to the analysis of the proficiency test sample were compared.

3.2 Proficiency tests and comparative tests

In 2007, RIKILT participated in two proficiency tests on PAHs. Relevant data are provided in Appendix 2. One proficiency test was organised by the CRL (IRMM, Geel, Belgium) on edible oil. The other one was organised by FAPAS and concerned olive oil. For determination of PAHs a confirmatory method based on GC-HRMS was used. The performance of RIKILT in the proficiency tests was good, Z-scores obtained were within -2 and +2, with exception of the determination of Cyclopenta[cd]pyrene.

3.3 Scientific and technical activities

The determination of priority PAHs is a challenging task. So far the two main analytical techniques used, GC-MS and HPLC-UV, do not allow accurate analysis. In GC there is a lack of separation between Benzo(B)fluoranthene, Benzo(K)fluoranthene, Benzo(J)fluoranthene. Therefore they can only be reported as a sum, whereas data on the individual compounds are required. With HPLC these PAHs can be separated, however acetonaftyleen can not be determined due to a lack of sensitivity in fluorescence detection. Therefore, within the CRL /NRL network alternative techniques have been discussed. It has been decided that in 2007/2008 the CRL would focus on the applicability of LC-MS (to improve detectability) while RIKILT would focus on the applicability of multi-dimensional GC (GCxGC-TOF-MS) to improve separation. Explorative experiments were performed in 2007, which showed that for proper separation of the PAHs, a more detailed study on the selection of column combination was required.

3.4 Plan for NRL activities 2008

RIKILT will finalise a pilot on the feasibility of GC x GC-TOF-MS. The first result will be presented during the CRL workshop in 2008 .

In 2008 RIKILT will participate in the following Proficiency tests:

- PAHs in edible oil organised by CRL Geel (Belgium)
- PAHs in meat organised by CRL Geel (Belgium)
- PAHs in edible and/or cod-liver oil organised by FAPAS (United Kingdom)

Participation in the workshops of the CRL
Organisation of 1-2 meetings with VWA Eindhoven and RIVM

3.5 References

[31] Commission regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories

3.6 Publications and presentations

Determination of PAHs using GPC and GC-HRMS, W. Traag, oral presentation, CRL workshop, Geel, February 2007.

4 Heavy metals

This chapter describes the activities of the NRL for heavy metals in feed according to EU document 882/2004 [41] and related activities in support of the expertise of RIKILT in this area. The focus is on the determination of Cd, As, Pb and Hg in fish caught in the wild, food of plant origin and animal feed.

4.1 CRL-NRL-OFL network

Table 3 CRL-NRL-OFL network for Heavy metals in food and feed

CRL [42]	The Joint Research Centre of the European Commission Geel, Belgium http://www.irmm.jrc.be/html/CRLs/crl_heavy_metals/index.htm	NRL	**feed** RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands W. A. Traag (MSc) Wim.traag@wur.nl **food** Laboratory of the Food and Consumer Product Safety Authority, Veldm. Montgomerylaan 500, 5623 LE Eindhoven, The Netherlands J. van der Wielen (MSc) Jacqueline.van.der.wielen@vwa.nl **food of animal origin [53]** National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven (PO Box 1, 3720 Bilthoven), The Netherlands Dr L.A. van Ginkel Leen.van.Ginkel@rivm.nl
OFL	Not applicable		

4.1.1 Participation in CRL workshops

During a workshop in September 2007 results of the proficiency test for the determination of Cd, Pb and Hg in mineral water have been presented. The results reported by RIKILT were well within the limits. Next to the outcome of the ring trial, half a day was spent on measurement uncertainty.

Shortly after the September workshop, samples of animal feed were sent out to participate in the NRL second proficiency test. The samples had to be extracted according to the standard operating procedure as well as a draft CEN method. The CEN method is based on boiling the sample with acid resulting in partial extraction.

4.1.2 NRL-OFL network

During a meeting mid 2007 information concerning methods for the determination of heavy metals in food and feed have been discussed with VWA Eindhoven. In 2008 standards and samples would be exchanged in order to compare results at the three laboratories.

4.2 Proficiency tests and comparative tests

In 2007, RIKILT participated in five proficiency tests on heavy metals. Relevant data are provided in Appendix 3. A proficiency test on fishmeal was organised by CRL (IRMM, Geel, Belgium). The other four were organised by FAPAS and concerned soya flour, canned tuna fish, canned crab meat and milk powder. The performance of RIKILT in the proficiency tests was good, Z-scores obtained were within -2 and +2, with exception of the determination of lead in flour where the reported value resulted in a Z-score of -2.5. After receiving the FAPAS report additional experiments were performed. The value that was reported too low was caused by matrix suppression. The soya flour samples were analysed again, this time using the standard addition method, giving much better results; calculation of the Z-score showed a value of -0.3. On the basis of this the FAPAS PT procedure was revisited; all samples with a calculated content of 50 % of the MRL have to be reanalysed with the standard addition method.

4.3 Scientific and technical activities

For the determination of Hg, a new system "Mecure" has been purchased. Using this new system measurements can be performed fully automated. The system combines a cold vapour technique with atom fluorescence. By combining both techniques a large dynamic range and low detection limits has been achieved. Also in 2007 an ICP-MS has been purchased giving possibilities for screening samples for a wide variety of elements in one run. The ICP-MS can be coupled with a HPLC system making the combination applicable for speciation. RIKILT focuses on speciation for As, Se and Hg.The first results will be reported in a RIKILT report

4.4 Plan for NRL activities 2008

RIKILT will finalize a study on speciation of mercury using LC-ICP-MS
Results will be discussed in 2008

In 2008 RIKILT will participate in the following Proficiency tests:

- As, Hg and Pb in animal feed organised by CRL Geel (Belgium)

Participation in the workshops of the CRL
Organisation of 1-2 meetings with VWA Eindhoven and RIVM

4.5 References

[41] 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, Official Journal of the European Union L 191, 28.5.2004, P 01-52.

[42] Commission regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories.

[43] Commission decision No 2006/130/EC of 10 February 2006 amending Decision 98/536/EC establishing the list of National Reference Laboratories for the detection of residues (notified under document number C(2006) 330).

[44] 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, Official Journal L 125 , 23/05/1996 P. 0010 – 0032.

5 Pesticides

This chapter describes the activities of the NRL for pesticides in cereals and feedingstuffs and products of animal origin and commodities with high fat content, according to EU document 882/2004 [51]. It also describes related activities in support of the expertise of RIKILT in this area. With respect to pesticides in products of animal origin, activities also cover those arising from the NRL task assigned to RIKILT [52] in the context of 96/23/EC [53] (group B2c substances/carbamates and pyrethroids; group B3a substances/organochlorine pesticides; group B3b substances/organophosphorus pesticides).

5.1 CRL-NRL-OFL network

Table 4 CRL-NRL-OFL 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 feedingstuffs	Fruits and vegetables, incl. commodities with high water and high acid content	Single residue methods
CRL [54]	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg Postfach 100462 D-79123 Freiburg Germany	National Food Institute Department of Food Chemistry Danish Technical University Moerkhoej Bygade 19 DK-2860 Soeborg Denmark	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	Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart Postfach 1206 D-70702 Fellbach Germany

	Food of animal origin and commodities with high fat content	Cereals and feedingstuffs	Fruits and vegetables, incl. commodities with high water and high acid content	Single residue methods
NRL	**organs/tissues animal products 96/23 B2c, B3a/b** [52] (carbamates/pyrethroids OCPs, OPPs) RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands Dr. J.G.J. Mol Hans.mol@wur.nl **food products** Laboratory of the Food and Consumer Product Safety authority, De Stoven 22, 7206 AX Zutphen, The Netherlands H. Van Rhijn (MSc) Hans.van.rhijn@vwa.nl	**feed** RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands Dr. J.G..J. Mol Hans.mol@wur.nl **food** Laboratory of the Food and Consumer Product Safety Authority, Hoogte Kadijk 401, 1018 BK Amsterdam, The Netherlands Dr. A. De Kok Andre.de.kok@vwa.nl	Laboratory of the Food and Consumer Product Safety Authority, Hoogte Kadijk 401, 1018 BK Amsterdam, The Netherlands Dr. A. De Kok Andre.de.kok@vwa.nl	Laboratory of the Food and Consumer Product Safety Authority, Hoogte Kadijk 401, 1018 BK Amsterdam, The Netherlands Dr. A. De Kok Andre.de.kok@vwa.nl
OFL	Not applicable	Not applicable	Not applicable	Not applicable

5.1.1 Participation CRL workshop

A joint workshop was organised by the four CRLs on pesticides in the context of 882/2004 (i.e. cereals/feed (CF), food of animal origin (AO), fruit/vegetables (FV), single residue methods (SRM)). The joint event was held on 26-29 September 2007 in Valencia, Spain and open for NRLs and laboratories involved in the official national and EU monitoring programs (OFLs). In total 160 people attended the workshop of which 71 were from NRLs.

Preceding the plenary program, the CRL-AO had a separate meeting on September 25. On this day the CRL-AO presented the set-up and preliminary results obtained for EUPT-AO2. From the 44 laboratories that participated, only 6 (RIKILT, 1 other NRL and 4 OFLs) covered the entire list of 50 pesticides that needed to be analysed. This indicates that the scope of most laboratories was insufficient. One reason for this might be that coordinated Community monitoring programs to ensure compliance with maximum levels of pesticide residues, up till now, focused on cereals and fruit/vegetables. In the Netherlands, and most other EU countries, monitoring of pesticide residues in products of animal origin is mainly done in the context of 96/23 [53] which only includes certain classes of pesticides (pyrethroids, organophosphorus pesticides, carbamates and organochlorine pesticides). The fact that monitoring of pesticides in products of animal origin is addressed in two different EU regulations (396/2005 [55] and 96/23) causes quite some confusion. It has also resulted in two different approaches with respect to validation and analytical quality control (AQC) in the field of pesticides residue analysis in food/feed

(i.e. products of animal origin *vs* products of plant origin). The latter was already recognised during the CRL-AO workshop on 4-5 December 2006. The general opinion was that validation and AQC procedures should be harmonised. Most participants considered a validation according to 2002/657 [56] (to be used in the context of 96/23) tedious and preferred a validation according to guideline SANCO/10232/2006.

Another topic addressed was the question "what are relevant pesticide residues in products of animal origin?". In contrast to fruit/vegetables and cereals, it was found to be very difficult to get data on residues in products of animal origin. This is partly due to a limited scope and low numbers of samples analysed (compared to fruit/vegetables), and partly because not all the generated data has been compiled into easily accessible formats. At the EU level, limited monitoring data on some pesticide residues can be found in EU staff working documents [57]. Other topics discussed during the CRL-AO meeting were the outcome of a questionnaire on analytical capabilities of the NRLs-AO and the CRL/NRL work program for 2008. In 2008 another proficiency test would be organised, this time in egg. Finally, an internet platform for the CRL-NRL network was presented that would be used for sharing of information.

The joint program started with a one day training program. Several presentations were held including one (by CRL-CF) on difficulties in GC-based analysis of pesticides in cereals, implementation of flexible scope in 17025 accreditation (CRL-FV) and using the Quechers method for determining acidic pesticides (CRL-SRM). The main topics of the workshop that took place in the following days were the outcome of all PTs organised by the CRLs in 2007, quality control of analytical reference standards, and the update of the guideline for validation and AQC of pesticide residues in food and feed (SANCO/10232/2006). The main issues on the AQC guideline were:

- to correct or not correct results for recovery;
- the re-classification of commodities groups and the inclusion of products of animal origin and;
- the number of pesticides to include in routine and periodical evaluation of recoveries and calibration.

In October 2007 the updated AQC guideline was issued (SANCO/3131/2007 [58])

During the plenary meetings the issue of harmonization of validation and AQC guidelines for pesticide residue analysis in products of plant and animal origin, which was initiated during the CRL-AO meeting, was further discussed with EU representatives (Luis Martin Plaza) who promised to discuss this further in DG-SANCO. Later in 2007 it was communicated through the CRL-AO that SANCO/3131/2007 in the near future will be accepted as "Lex specialis" which in practice means that for pesticide residue analysis validation can be done according to SANCO/3131/2007 (i.e. 96/23 no longer applies). During the current transition period, the requirement to have methods for pesticide residues in animal products validated according to 96/23 before September 2007 will not be enforced.

5.1.2 NRL-OFL interactions

One of the tasks of the NRL is to communicate with the Competent Authority, Official Field Laboratories and other NRLs on issues regarding the control of levels of pesticide residues. Since for the determination of pesticide residues no OFLs are operational within the Netherlands, the

communication and cooperation is restricted to the pesticide NRLs on food (VWA laboratories in Amsterdam [FC, FV, SRM] and Zutphen [AO]). Three meetings were held in 2007. The main purpose of these meetings was to coordinate NRL tasks, and to discuss analytical matters in order to have a joined opinion on issues to be discussed in CRL meetings (e.g. quality control, analytical methods).

With VWA Amsterdam, analytical reference solutions were exchanged for verification of accuracy. For most pesticides the concentration was correct but in a limited number of cases deviations were observed. In such cases new standard materials were purchased and further verification was performed.

Although there are no OFLs, RIKILT exchanged information and interacted with laboratories offering their services to the food/feed industry. RIKILT is chairing the user group for proficiency testing on organochlorine pesticides and PCBs, 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 organisers. RIKILT followed up to a dispute regarding a MRL violation of endosulfan in a feed ingredient (oil product). The sample was analysed as part of the official monitoring program. Upon reporting the violation, the producer demanded a contra-analysis, which was performed at a commercial laboratory (ISO17025 accredited). Initially, the laboratory was not able to detect any endosulfan. After exchange of information and repeated analysis, the MRL violation was confirmed.

5.2 Proficiency tests and comparative tests

Within the domain of pesticide residues in cereals, products of animal origin and commodities with high fat content, RIKILT participated in 5 proficiency tests covering a variety of pesticides. Relevant data are provided in Appendix 4. Acceptable quantitative laboratory performance was achieved in 19 out of 20 cases. The reason for the one deviating Z-score was an inaccurate analytical reference standard, which emphasises the importance of cross-checking of such materials as described under 5.1.2. From the data in Appendix 4, it can be seen that for products with high fat content (oil) and also in minced chicken, the inter-laboratory reproducibility is high (often >25% relative standard deviation), indicating that analysis results from laboratories vary considerably for this type of analyte/matrix combinations. In certain cases, the variation in results was too high to establish a reliable assigned value. Consequently, Z-scores could not always be provided. In comparison with the proficiency test for vegetables/fruits, pesticide residue analysis in products with high fat content seems to be more challenging and certainly needs to be improved among the laboratories in the EU.

5.3 Scientific and technical activities

Within the framework of the NRL task and also as part of other projects, upcoming new analytical techniques and approaches are considered and studied were appropriate. The aim is improvement of the reliability, efficiency and, if necessary, sensitivity of methods for determination of pesticides in cereals/feed and products of animal origin.

In 2007, research was done on three topics:

1) *Cereal/feed analysis by GCxGC-TOF-MS.*

In the past years a lot of effort was put into the development of multi-dimensional gas chromatography with time-of-flight mass spectrometric detection (GCxGC-TOF-MS). This technique is very suitable for wide-scope screening of GC-amenable pesticides residues in very complex matrices like animal feed. So far, there were doubts about the quantitative performance of this approach. In 2007 the sample preparation has been modified (inclusion of dispersive SPE clean up step) and the overall procedure has been validated for approx. 100 pesticides in a complex cereal-based feed matrix. The results have been published in a peer-reviewed paper[5a] and presented at international symposia[5b,5c].

2) *Comparison of analytical methods for cereal/feeds and products with high fat content*

RIKILT has a range of analytical techniques available for determination of pesticide residues, which include GC-ECD, GC-MS/MS, GCxGC-TOF-MS and LC-MS/MS. The techniques differ in sensitivity, selectivity, scope and ease of use. The samples from the proficiency tests were used to compare the different approaches. In most cases, the results obtained with the different techniques were in agreement with each other. GCxGC-TOF-MS was found to be very useful for expanding the scope of analysis and for pre-screening samples for a wide range of pesticides, although data handling is not very straightforward. With the classical GC-ECD method acceptable results could be obtained for organochlorine pesticides and PCBs in vegetable oil and animal fat. However, for feed and mixed/processed fat, the selectivity of the method was often insufficient. In that case, GC-MS/MS was found to be more useful. For a number of polar compounds the quantitative performance was found to be better when using LC-MS/MS instead of a GC-based technique. The final conclusion was that GCxGC-TOF-MS is very suitable for simultaneous screening and quantitative analysis. GC-MS/MS is an alternative with a narrower scope but easier data evaluation. For compounds that are amenable to LC-MS/MS, this is the preferred technique for quantitative analysis. GC-ECD is only useful for screening a limited number of chlorinated analytes in non-complex samples.

3) *Generic extraction method for pesticides (and other residues/contaminants) in cereals/feed and products of animal origin*

In pesticide residue analysis, emphasis has always been on vegetables/fruit, and to a lesser extent cereals/food. Much less attention has been paid to feed and products of animal origin. Therefore, research has been conducted to verify the applicability of existing pesticide multi-residue methods, once developed for vegetables/fruit, for the analysis of feed (compound feed, maize) and animal products (tissue, milk, egg, honey). Since in such commodities also other residues and contaminants are of interest, this work was integrated into a larger project to develop a generic extraction method that allows efficient extraction not only of pesticides but also natural toxins (mycotoxins, plant toxins) and veterinary drugs. Several existing multi-methods and three new approaches were evaluated. From the method comparison, two methods were found to be suitable as generic multi-analyte/multi-matrix methods. In 2008 these methods would be fine-tuned and validated. Preliminary results were presented at several symposia and workshops (see publications and presentations [5d-5f]).

5.4 Plan for NRL activities 2008

In 2008 RIKILT will attend the annual CRL workshops on Pesticides in cereals/feed (Copenhagen) and on pesticide in animal products (Freiburg). Meetings with the NRL pesticides in cereals/food and products of animal origin/consumer products will be held, similar as in 2007. Analytical standards will be exchanged with the NRL pesticides/food and/or other laboratories for further verification of accuracy. RIKILT will participate in a number of proficiency tests organised by the CRL and FAPAS. Scientific activities will focus on simplification of methods for products with high fat content. In addition, generic methods developed in 2007 will be fine-tuned and validated. Where relevant, RIKILT will attend symposia, workshops, and CEN meetings.

5.5 References

[51] 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, Official Journal of the European Union L 191, 28.5.2004, P 01-52.

[52] Commission decision No 2006/130/EC of 10 February 2006 amending Decision 98/536/EC establishing the list of National Reference Laboratories for the detection of residues (notified under document number C(2006) 330).

[53] 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, Official Journal L 125 , 23/05/1996 P. 0010 – 0032.

[54] Commission regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories.

[55] 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, Official Journal of the European Union L 70, 16.3.2005, P 01-16.

[56] 2002/657, Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities L221, 17.8.2002, P 08-36.

[57] Commission staff working document on the implementation of national residue monitoring plans in the member states in 2005, Brussels, 8.2.2007, SEC(2007) 196
http://ec.europa.eu/food/food/chemicalsafety/residues/workdoc_2005_en.pdf

[58] Method validation and quality control procedures for pesticide residue analysis in food and feed, Document N° SANCO/2007/3131, 31/October/2007 (Supersedes Document No. SANCO/10232/2006).

5.6 Publications and presentations

Peer reviewed papers:

[5a] M.K. van der Lee, G. van der Weg, W.A. Traag, H.G.J. Mol, "Qualitative screening and quantitative determination of pesticides and contaminants in animal feed using comprehensive two dimensional gas chromatography with time-of-flight mass Spectrometry" J. Chromatogr. A, 1186 (2008) 325–339.

Contributions at Conferences and Workshops:

[5b] Generic pesticide/contaminant analysis in food and feed using GC and LC with mass spectrometric detection, H. Mol, P. Zomer, M. v.d. Lee, G. v.d. Weg, Th. de Rijk, W. Traag, lecture at 4th International Fresenius Conference Pesticide Residues in Food, 9-10 July 2007, Frankfurt, Germany.

[5c] Cereals/feed analyses based on ethyl acetate extraction followed by GCxGC-TOF-MS, H.Mol, M. van der Lee, G. van der Weg, W. Traag, lecture at 7th NPRW2007, Uppsala, Sweden, 19-20 November 2007.

[5d] Combined determination of mycotoxins, plant toxins and pesticides in animal feed using generic extraction and UPLC with mass spectrometric detection, H. Mol, P. Zomer, Th. de Rijk, P. Mulder, lecture at ICC Workshop "The safety of animal feed and its contribution to the human food chain", Glasgow, UK, 2-5 September 2007.

[5e] Development of a UPLC-TOF-MS Method for Combined Determination of Pesticides, Mycotoxins and Plant toxins in Food and Feed, P. Zomer, P. Plaza Bolaños, Th.de Rijk, P. Mulder, H. Mol, poster at 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November, Prague, Czech Republic.

[5f] Comprehensive screening of organic contaminants in the food chain using UPLC or GC(xGC) and full scan mass spectrometric detection, H. Mol, P. Zomer, P. Plaza-Bolaños, M. vd Lee, G. vd Weg, W. Traag, L. Stolker, P. Mulder, lecture at 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November, Prague, Czech Republic.

6 Mycotoxins

This chapter describes the activities of the NRL for mycotoxins in feed according to EU document 882/2004 [61]and related activities in support of the expertise of RIKILT in this area. A separate CRL [62] and NRL [63] have been appointed in the context of 96/23/EC [64], which focuses on the determination of mycotoxins in products of animal origin. Concerning the latter, RIVM is both CRL and NRL. These activities are not included in this report.

6.1 CRL-NRL-OFL network

Table 5 CRL-NRL-OFL network for Mycotoxins in food and feed

CRL [62]	The Joint Research Centre of the European Commission Geel, Belgium http://www.irmm.jrc.be/html/CRLs/crl_mycotoxins/index.htm	NRL	**feed** RIKILT – Institute of Food Safety Bornsesteeg 45, 6708 PD Wageningen, The Netherlands Dr. J.G.J. Mol Hans.mol@wur.nl **food** Laboratory of the Food and Consumer Product Safety Authority, Hoogte Kadijk 401, 1018 BK Amsterdam, The Netherlands Dr. A. De Kok Andre.de.kok@vwa.nl **food of animal origin [62]** National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven (PO Box 1, 3720 Bilthoven), The Netherlands S.S. Sterk (MSc) Saskia.Sterk@rivm.nl
OFL	Not applicable		

6.1.1 Participation in CRL workshop

The second CRL workshop organised by the CRL (IRMM, Geel, Belgium) was held on 22-23 March in Geel, Belgium. Delegates from 24 EU countries and 4 accession countries were present. Several countries were represented by multiple delegates. For the Netherlands, delegates from RIKILT (feed) and VWA (food) were present. The session started with an overview of news relevant to the CRL-NRL network. This included the presentation of a website (outcome of the CRL meeting on 13 February 2007

with DG SANCO) and the presentation of the role of the CRL to initiate corrective action in case of recurrent underperformance of the NRL, where necessary. Following this, the outcome of a proficiency test conducted within the CRL/NRL network in November 2006 was presented and discussed. The Netherlands had not participated in this test because at that time the NRLs from the Netherlands were not yet known by the CRL. The correctness of calibrants and way of calibration were found to be important factors in the overall variability of analytical results. Protection from (UV) light and conditioning of glassware were recognized as critical parameters.
Ronald Schothorst (RIVM, The Netherlands) gave a presentation on the SCOOP[1] task on Fusarium toxins. Most relevant conclusions were:

- among cereals, corn shows the highest level of contamination with trichothecenes;
- lower limits of detection are required for assessment of intake;
- reference materials should be developed and;
- there is a lack of harmonization with respect to sampling procedures, analytical methods and quality assurance.

Further, the results of an interlaboratory validation on T2 and HT2-toxins in which RIKILT participated, were discussed (also see below). Finally, the work program for the following year was presented which includes a follow up proficiency test for aflatoxins in peanut butter.

6.1.2 NRL-OFL interactions

One of the tasks of the NRL is to communicate with the Competent Authority, Official Field Laboratories and other NRLs on issues regarding the control of levels of mycotoxins. Since for the determination of mycotoxins no OFLs are operational within the Netherlands, the communication and cooperation is restricted to the NRL food. Three meetings were held in 2007. The main purpose of these meetings was to coordinate NRL tasks, to discuss analytical matters in order to have a joined opinion on issues to be discussed in CRL meetings (e.g. quality control, analytical methods). Furthermore the content of the monitoring programs and trends in contamination of feed and food were discussed.

6.2 Proficiency and comparative tests

In 2007, RIKILT participated in nine proficiency tests with varying mycotoxins, mostly in feed related matrices. Relevant data are provided in Appendix 5. For determination of mycotoxins, one multi-method based on LC-MS/MS is used. With the exception of patulin, this method covers all mycotoxins for which MRLs or recommended limits have been established within the EU[65,66]. For aflatoxins, in addition to the multi-method also the classical method based on immuno-affinity clean up and LC with fluorescence detection was used to ensure accurate quantitative determination at sub-µg/kg levels. In general the performance of RIKILT in the proficiency tests was good, i.e. 22 out of 23 Z-scores obtained were within -2 and +2, indicating adequate quantitative laboratory performance. Follow up was given to find the cause of the one deviating result and to prevent re-occurrence.

The data in Appendix 5 also provide indicative information on the precision typically obtained with this type of analysis. The relative standard deviation of the robust mean calculated from the results from all

[1] Scientific CO-OPeration on questions relating to food

labs varied between 16 and 42%, with an average of 28%. This implies a typical expanded measurement uncertainty of 56% (ranging from 32-84%).

RIKILT participated in an interlaboratory comparison test for the determination of T2 and HT2-toxin in animal feed, baby food and breakfast cereals. A prescribed method was supplied by the CRL together with reference standards, 13C-labeled internal standards and immuno-affinity columns for clean up. The method involved an extraction with methanol/water, clean up using immuno-affinity chromatography, derivatisation with TMS-reagent and GC-MS analysis. The interlab reproducibility (RSD(R)) was in the range of 12-30% and the Horrat ratio between 0.5 and 1.6 with one exception. The same samples were used to compare the GC-MS based method from the CRL with the LC-MS/MS based multi-method from RIKILT. Comparable results were obtained.

6.3 Scientific and technical activities

Within the framework of the NRL task and also as part of other projects, upcoming new analytical techniques and approaches are considered and studied where appropriate. The aim is improvement of the reliability, efficiency and, if necessary, sensitivity of methods for determination of mycotoxins in feed.

In 2007, research was done on three topics:

1) *Multi-compound immunoaffinity clean up columns*
   The LC-MS/MS multi-method for mycotoxins in feed is very efficient because all mycotoxins that have been regulated can be determined by one method. However, the method involves a generic extraction based on acetonitrile/water and for compound feed very complex extracts are obtained. Although LC-MS/MS can deal with such extracts in terms of selectivity, the response of the mycotoxins is suppressed and matrix dependent. In order to allow reliable quantitative determination, calibration using the standard-addition method needs to be employed. In addition, the required sensitivity for aflatoxins can not always be achieved and in such cases samples need to be re-analysed using a separate dedicated method. One way of solving these drawbacks of the multi-mycotoxin method is to incorporate a clean up using mixed immunoaffinity columns (IAC). Such columns are being developed by two manufacturers with whom RIKILT cooperates as key applicant. The multi-IAC developed by one manufacturer for DON, T2, HT2 and Zearalenon was found to be suited for feed analysis. The other multi-IAC included more mycotoxins (Aflatoxine B1, Fumonisine B1/B2/B3, T2 en HT2, Deoxynivalenol, Ochratoxine and Zearalenon) but recovery, reproducibility and robustness did not meet the quality control criteria. Feedback was provided to the manufacturers which will use the information to improve their multi-IACs.

2) *Use of UPLC-TOF-MS for determination of mycotoxins*
   UPLC (ultra performance liquid chromatography) basically is an improved version of HPLC; better separation and/or higher analysis speed can be achieved by using special LC columns with smaller particles. Another advantage is that narrower peaks are obtained which improves sensitivity (S/N). TOF-MS (time-of-flight mass spectrometry) is an emerging MS detection technique allowing full scan acquisition. The advantage is that there is no need to decide beforehand which analytes need to be determined. After the analysis, specific signals for

mycotoxins can be extracted from the raw data, not only for regulated mycotoxins, but also for other natural toxins that are of interest. This can also be done retrospectively. A method was set up to explore the possibilities. In addition, a comparison was made with UPLC-MS/MS detection. The latter proved to be better suited in terms of sensitivity and selectivity and, therefore, remains the preferred technique for quantitative determination of a limited number of pre-known compounds. The results on UPLC-TOF-MS were presented at an international symposium[6a]. During this symposium, RIKILT also attended the meeting the of CEN workinggroup CEN/TC 275/WG 5 Biotoxins.

3) *Combined determination of mycotoxins with other residues and (natural) contaminants*
Besides mycotoxins, a number of other compounds like plant toxins and pesticide residues may occur in feedingstuff and are being monitored. Currently, this is done using a range of different multi- and single-compound methods. It would be more efficient to combine the determination of all these residues and contaminants in one method. A uniform generic extraction protocol and uniform conditions for UPLC-MS/MS or TOF-MS are required for this. In 2007 several existing and new extraction procedures have been compared for extraction of mycotoxins in feed and maize. Furthermore, different conditions (eluents) have been evaluated for separation and optimal response of mycotoxins. The results have been presented at several symposia and workshops[6b-e].

6.4 Plan for NRL activities 2008

In 2008 RIKILT will attend the annual CRL workshop (March 2008). In addition, RIKILT will organise a session with the CRL to share experiences on the use of LC-MS/MS and generic extraction methods in mycotoxin analysis and discuss analytical quality control and measurement uncertainty in more detail. Meetings with the NRL mycotoxins in food will be held, similar as in 2007. RIKILT will participate in a number of proficiency tests organised by the CRL and FAPAS. Scientific activities will focus on continuation of the cooperation with manufacturers of multi-IACs, extension of scope and use of stable isotope dilution as an option to deal with matrix effects. In addition, generic methods developed in 2007 will be fine-tuned and validated. Where relevant, RIKILT will attend symposia, workshops, and CEN meetings.

6.5 References

[61] 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, Official Journal of the European Union L 191, 28.5.2004, P 01-52.

[62] Commission regulation (EC) No 776/2006 of 23 May 2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards Community Reference Laboratories.

[63] Commission decision No 2006/130/EC of 10 February 2006 amending Decision 98/536/EC establishing the list of National Reference Laboratories for the detection of residues (notified under document number C(2006) 330).

[64] 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, Official Journal L 125 , 23/05/1996 P. 0010 – 0032.

[65] Commission regulation(EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[66] Commission recommendation No 2006/576/EC of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding.

6.6 Publications and presentations

Contributions at Conferences and Workshops:

[6a]The analysis of mycotoxins in animal feed with UPLC-Time-Of -Flight Mass Spectrometry, T.C. de Rijk, M.C. van Engelen, W.A. Traag, Poster 1475 / Netherlands at the XII International IUPAC Symposium on Mycotoxins and Phycotoxins, May 21-25, 2007, Istanbul, Turkey,

[6b]Generic pesticide/contaminant analysis in food and feed using GC and LC with mass spectrometric detection, H. Mol, P. Zomer, M. v.d. Lee, G. v.d. Weg, Th. de Rijk, W. Traag, lecture at 4th International Fresenius Conference Pesticide Residues in Food, 9-10 July 2007, Frankfurt, Germany

[6c]Combined determination of mycotoxins, plant toxins and pesticides in animal feed using generic extraction and UPLC with mass spectrometric detection, H. Mol, P. Zomer, Th. de Rijk, P. Mulder, lecture at ICC Workshop “The safety of animal feed and its contribution to the human food chain”, Glasgow, UK, 2-5 September 2007

[6d]Development of a UPLC-TOF-MS Method for Combined Determination of Pesticides, Mycotoxins and Plant toxins in Food and Feed, P. Zomer, P. Plaza Bolaños, Th.de Rijk, P. Mulder, H. Mol, poster at 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November, Prague, Czech Republic

[6e]Comprehensive screening of organic contaminants in the food chain using UPLC or GC(xGC) and full scan mass spectrometric detection, H. Mol, P. Zomer, P. Plaza-Bolaños, M. vd Lee, G. vd Weg, W. Traag, L. Stolker, P. Mulder, lecture at 3rd International Symposium on Recent Advances in Food Analysis, 7-9 November, Prague, Czech Republic

Appendix 1 Dioxins and PCBs

Overview of relevant data from proficiency tests on dioxins and PCBs in which RIKILT participated in 2007.

Analyte	Matrix	amount spiked in µg/kg	consensus value in µg/kg	# labs consensus	target RSD% used for calculation of Z-score	precision (%RSD) from prof. test	Z-score * RIKILT	organiser
PCB indicator sum (6)	salmon	incurred	3.78		20		-0.6	NIPH**
PCB indicator sum (6)	chicken meat	incurred	21.4		20		-1.3	NIPH
PCB indicator sum (6)	butter	incurred	3.72		20		-0.8	NIPH
PCB-101	veg. oil	50	42.1	16	22	22	0.2	Fapas
TEQ mono-ortho PCB	salmon	incurred	0.00013		20		0.5	NIPH
TEQ mono-ortho PCB	chicken meat	incurred	0.00084		20		0.2	NIPH
TEQ mono-ortho PCB	butter	incurred	0.00012		20		0.2	NIPH
TEQ mono-ortho PCB lower	cod liver oil	incurred	0.00196	20	22	11	0.4	Fapas
TEQ mono-ortho PCB upper	cod liver oil	incurred	0.00196	20	22	11	0.4	Fapas
TEQ non-ortho PCB	salmon	incurred	0.00042		20		1.3	NIPH
TEQ non-ortho PCB	chicken meat	incurred	0.00025		20		1.0	NIPH
TEQ non-ortho PCB	butter	incurred	0.00076		20		1.3	NIPH
TEQ non-ortho PCB lower	cod liver oil	incurred	0.00171	23	22	26	0.3	Fapas
TEQ non-ortho PCB upper	cod liver oil	incurred	0.00181	24	22	27	0.0	Fapas
TEQ PCDD/PCDF	salmon	incurred	0.00012		20		0.0	NIPH
TEQ PCDD/PCDF	chicken meat	incurred	0.00051		20		0.2	NIPH
TEQ PCDD/PCDF	butter	incurred	0.00038		20		-0.6	NIPH
TEQ PCDD/PCDF lower	cod liver oil	incurred	0.0006	27	22	28	0.1	Fapas
TEQ PCDD/PCDF upper	cod liver oil	incurred	0.00064	28	22	25	-0.2	Fapas
TEQ total	salmon	incurred	0.00066		20		1.0	NIPH
TEQ total	chicken meat	incurred	0.0016		20		0.3	NIPH
TEQ total	butter	incurred	0.0013		20		0.6	NIPH
fat content (%)	salmon	incurred	12.1		20		0.6	NIPH
fat content (%)	chicken meat	incurred	7.75		20		0.2	NIPH
fat content (%)	butter	incurred	83		20		-0.2	NIPH

* Z-scores: between -2 and 2 means adequate performance, between -3 and -2 or 2 and 3 means: questionable performance; < -3 or > 3 means bad performance

**NIPH = Norwegian Institute of Public Health (Folkehelseinstituttet)

Appendix 2 Polycyclic Aromatic Hydrocarbons (PAHs)

Overview of relevant data from proficiency tests on PAHs in which RIKILT participated in 2007.

Analyte	Matrix	Amount spiked in µg/kg	consensus value in µg/kg	# labs consensus (%)	target RSD% used for claculation of z-score	Z score *6 RIKILT	Organiser
Benzo[c]fluorene	Olive oil	spike *1	0.87	86	22	-0.1	FAPAS 0635
Benzo[a]anthracene	Olive oil	spike *1	3.17	82	22	0.3	FAPAS 0635
Cyclopenta[cd]pyrene	Olive oil	spike *1	0.54	47	22	**6.4** *2	FAPAS 0635
Chrysene	Olive oil	spike *1	9.52	86	22	0.4	FAPAS 0635
5-Methylchrysene	Olive oil	spike *1	NA				FAPAS 0635
Benzo[b]fluoranthene	Olive oil	spike *1	2.40	83	22	0.4	FAPAS 0635
Benzo[j]fluoranthene	Olive oil	spike *1	1.22	71	22	-0.1	FAPAS 0635
Benzo[k]fluoranthene	Olive oil	spike *1	0.98	86	22	0.1	FAPAS 0635
Benzo[a]pyrene	Olive oil	spike *1	2.06	89	22	0.3	FAPAS 0635
indeno[123cd]pyrene	Olive oil	spike *1	1.61	83	22	0.0	FAPAS 0635
Dibenzo[ah]anthracene	Olive oil	spike *1	2.15	92	22	0.1	FAPAS 0635
Benzo[ghi]perylene	Olive oil	spike *1	1.54	82	22	-0.1	FAPAS 0635
Dibenzo[a,l]pyrene	Olive oil	spike *1	1.47	93	22	-0.2	FAPAS 0635
Dibenzo[a,e]pyrene	Olive oil	spike *1	1.52	89	22	-0.1	FAPAS 0635
Dibenzo[a,i]pyrene	Olive oil	spike *1	1.21	83	22	-0.8	FAPAS 0635
Dibenzo[a,h]pyrene	Olive oil	spike *1	1.50	67	22	-0.6	FAPAS 0635
Benzo[c]fluorene	Edible oil	spike *3	7.40	*4	22	0.1	CRL (IRMM, Geel)
Benzo[a]anthracene	Edible oil	spike *3	1.20		22	0.5	CRL (IRMM, Geel)
Cyclopenta[cd]pyrene	Edible oil	spike *3	6.30		22	-1.1	CRL (IRMM, Geel)
Chrysene	Edible oil	spike *3	2.20		22	-0.1	CRL (IRMM, Geel)
5-Methylchrysene	Edible oil	spike *3	3.30		22	-0.1	CRL (IRMM, Geel)
Benzo[b]fluoranthene	Edible oil	spike *3	2.60		22	*5	CRL (IRMM, Geel)
Benzo[j]fluoranthene	Edible oil	spike *3	7.10		22	*5	CRL (IRMM, Geel)
Benzo[k]fluoranthene	Edible oil	spike *3	4.20		22	0	CRL (IRMM, Geel)
Benzo[a]pyrene	Edible oil	spike *3	1.40		22	-0.2	CRL (IRMM, Geel)
indeno[123cd]pyrene	Edible oil	spike *3	8.70		22	-0.1	CRL (IRMM, Geel)
Dibenzo[ah]anthracene	Edible oil	spike *3	7.00		22	0	CRL (IRMM, Geel)
Benzo[ghi]perylene	Edible oil	spike *3	5.40		22	-0.1	CRL (IRMM, Geel)
Dibenzo[a,l]pyrene	Edible oil	spike *3	1.70		22	1.2	CRL (IRMM, Geel)
Dibenzo[a,e]pyrene	Edible oil	spike *3	2.80		22	0.1	CRL (IRMM, Geel)
Dibenzo[a,i]pyrene	Edible oil	spike *3	6.20		22	-0.6	CRL (IRMM, Geel)
Dibenzo[a,h]pyrene	Edible oil	spike *3	4.60		22	-0.3	CRL (IRMM, Geel)

NA = Not Assigned
*1 Spike level ranging from 0.03 - 10 µg/kg
*2 Cyclopenta[cd]pyrene is not well separated and interfered using GC-MS; This compound is not included in the RIKILT method
*3 Spike level ranging from 1 - 10 µg/kg
*4 60 -80 % of all results were within a range of 10% from the assigned value
* 5 not reported by RIKILT
*6 Z-scores: between -2 and 2 means adequate performance, between -3 and 3 menas; questionable performance; < -3 or > 3 means bad performance

Appendix 3 Heavy metals and arsenic

Overview of relevant data from proficiency tests on heavy metals and arsenic in which RIKILT participated in 2007.

Analyte	Matrix	amount spiked in µg/kg	consensus value in µg/kg	# labs consensus	target RSD% used for calculation of Z-score	precision (%RSD) from prof. test	Z-score * RIKILT	organiser
arsenic (total)	soya flour	incurred + spike	436	53	18	18	-1	Fapas
arsenic (total)	fish, tuna, canned	incurred	738	33	17	13	-1	Fapas
arsenic (total)	fish, swordfish steak, cann	?	1124	82	10	19	-1.9	Fapas
arsenic (total)	milk powder	spike/incurred (18)	121	82	22	36	not avail.	Fapas
arsenic (total)	crab meat, canned	incurred	11400	45	11	11	-0.8	Fapas
cadmium	feed (fish meal)	incurred	1.1	1	16		0.5	CRL (IRMM, Geel)
cadmium (5% HNO3)	feed (fish meal)	incurred	1.1	1	16		-0.6	CRL (IRMM, Geel)
cadmium	fish, swordfish steak, cann	?	52.4	123	22	17	-0.4	Fapas
cadmium	milk powder	spike	52.7	123	22	19	-1.2	Fapas
cadmium	soya flour	incurred + spike	235	64	20	17	0.2	Fapas
cadmium	crab meat, canned	incurred	5810	61	12	8	-0.4	Fapas
lead	soya flour	incurred + spike	367	63	19	28	-2.5***	Fapas
lead	feed (fish meal)	incurred			22		**	CRL (IRMM, Geel)
lead (5% HNO3)	feed (fish meal)	incurred			22		**	CRL (IRMM, Geel)
lead	fish, swordfish steak, cann	?					**	Fapas
lead	milk powder	spike/incurred (6)	84.5	134	22	31	-1.5	Fapas
mercury (total)	soya flour	incurred + spike	231	59	20	25	0.7	Fapas
mercury (total)	feed (fish meal)	incurred	49.9	1	22		-0.2	CRL (IRMM, Geel)
mercury (total)	fish, tuna, canned	incurred	53.4	50	22	21	0.4	Fapas
mercury (total)	fish, swordfish steak, cann	?	704	134	17	22	0.4	Fapas
mercury (total)	milk powder	spike	72.3	99	22	26	0.4	Fapas
mercury (total)	crab meat, canned	incurred	97.9	58	22	15	0.8	Fapas

* Z-scores: between -2 and 2 means adequate performance, between -3 and -2 or 2 and 3 means: questionable performance; < -3 or > 3 means bad performance
** no Z-score provided
*** Z-score of 0.3 after corrective action

Appendix 4 Pesticides

Overview of relevant data from proficiency tests on pesticides in cereals and products of animal origin/high fat content in which RIKILT participated in 2007.

Analyte	Matrix	amount spiked in µg/kg	consensus value in µg/kg	# labs consensus	target RSD% used for calculation of Z-score	precision (%RSD) from prof. test	Z-score * RIKILT	organiser	code
DDD, pp'	chicken, minced	60	40.4	12	22	52	**	Fapas	555
DDE, pp'	chicken, minced	60	39.2	14	22	55	**	Fapas	555
DDT, op'	chicken, minced	50	27.4	9	22	42	**	Fapas	555
DDT, pp'	chicken, minced	120	72.7	13	22	49	**	Fapas	555
HCH, beta	chicken, minced	90	57.3	12	22	58	**	Fapas	555
HCH, gamma	chicken, minced	80	59.7	14	22	50	**	Fapas	555
bifenthrin	oil/fat, vegetable	80	71	not avail.	25	24	-1.1	CRL PEST AO	EUPT AO 02
chlorpyriphos-methyl	oil/fat, vegetable	60	52.2	not avail.	25	32	-1.2	CRL PEST AO	EUPT AO 02
deltamethrin	oil/fat, vegetable	60	57	not avail.	25	63	**	CRL PEST AO	EUPT AO 02
dieldrin	oil/fat, vegetable	90	79.9	not avail.	25	23	0.0	CRL PEST AO	EUPT AO 02
fenvalerate (sum all isomers)	oil/fat, vegetable	50	54	not avail.	25	26	0.0	CRL PEST AO	EUPT AO 02
HCH, gamma	oil/fat, vegetable	26	21.9	not avail.	25	29	-0.7	CRL PEST AO	EUPT AO 02
heptachlor epoxide, cis	oil/fat, vegetable	50	45	not avail.	25	20	-0.2	CRL PEST AO	EUPT AO 02
methacrifos	oil/fat, vegetable	100	85.3	not avail.	25	28	0.5	CRL PEST AO	EUPT AO 02
parathion	oil/fat, vegetable	50	47.8	not avail.	25	28	-0.7	CRL PEST AO	EUPT AO 02
pirimiphos-methyl	oil/fat, vegetable	100	92.4	not avail.	25	39	5.4***	CRL PEST AO	EUPT AO 02
quintozene	oil/fat, vegetable	20	18.2	not avail.	25	25	-0.7	CRL PEST AO	EUPT AO 02
resmethrin (sum isomers)	oil/fat, vegetable	110	80.3	not avail.	25	47	0.1	CRL PEST AO	EUPT AO 02
azinphos-methyl	oil/fat, vegetable, hydrolysed	300	(255)	10	20	26	**	Fapas	947
chlorpyrifos	oil/fat, vegetable, hydrolysed	80	73.4	20	22	22	0.4	Fapas	947
cyhalothrin, lambda	oil/fat, vegetable, hydrolysed	250	206	14	20	24	**	Fapas	947
diazinon	oil/fat, vegetable, hydrolysed	140	123	20	22	22	1.1	Fapas	947
etrimfos	oil/fat, vegetable, hydrolysed	200	(150)	14	21	40	**	Fapas	947
HCH, alpha	oil/fat, vegetable, hydrolysed	70	65.8	26	22	23	-0.3	Fapas	556
HCH, gamma	oil/fat, vegetable, hydrolysed	30	28.8	24	22	25	-0.7	Fapas	556
azoxystrobin	wheat	incurred	240	not avail.	25	not available	-0.1	CRL PEST cereals	EUPT-C1-SRM-2
carbendazim	wheat	incurred/spiked	126	not avail.	25	not available	-0.4	CRL PEST cereals	EUPT-C1-SRM-2
deltamethrin	wheat	incurred/spiked	342	not avail.	25	not available	1.1	CRL PEST cereals	EUPT-C1-SRM-2
diazinon	wheat	incurred	78	not avail.	25	not available	0.5	CRL PEST cereals	EUPT-C1-SRM-2
endosulfan	wheat	incurred	not avail.	not avail.	not available	not available	**	CRL PEST cereals	EUPT-C1-SRM-2
pirimiphos-methyl	wheat	incurred	6330	not avail.	25	not available	0.6	CRL PEST cereals	EUPT-C1-SRM-2
propiconazole	wheat	spiked	353	not avail.	25	not available	-0.4	CRL PEST cereals	EUPT-C1-SRM-2

* Z-scores: between -2 and 2 means adequate performance, between -3 and -2 or 2 and 3 means: questionable performance; < -3 or > 3 means bad performance
** no Z-score provided
*** Z-score of 0.3 after corrective action

Appendix 5 Mycotoxins

Overview of relevant data from proficiency tests on mycotoxins in which RIKILT participated in 2007.

Analyte	Matrix	amount spiked in µg/kg	consensus value in µg/kg	# labs consensus	target RSD% used for calculation of Z-score	precision (%RSD) from prof. test	Z-score * RIKILT	organiser
aflatoxin B1	peanut	incurred	1.77		22	not available	-0.5	CRL (IRMM, Geel)
aflatoxin B1	feed, nut based	spike	18.1	34	22	16	1.4	Fapas
aflatoxin B1	feed, cereal based	spike	7.29	51	22	27	0.3	Fapas
aflatoxin B2	peanut	incurred	0.48		22	not available	-0.3	CRL (IRMM, Geel)
aflatoxin B2	feed, nut based	spike	1.51	25	22	29	1.0	Fapas
aflatoxin B2	feed, cereal based	spike	1.69	46	22	35	-0.1	Fapas
aflatoxin G1	peanut	incurred	0.92		22	?	-0.7	CRL (IRMM, Geel)
aflatoxin G1	feed, nut based	spike	5.86	26	22	24	0.8	Fapas
aflatoxin G1	feed, cereal based	spike	6.43	47	22	32	-0.3	Fapas
aflatoxin G2	peanut	incurred	0.31		22	not available	-0.9	CRL (IRMM, Geel)
aflatoxin G2	feed, nut based	spike	< LOQ		22	not available	**	Fapas
aflatoxin G2	feed, cereal based	spike	1.06	42	22	42	0.1	Fapas
aflatoxin M1	milk powder	spike	0.323	41	22	35	0.1	Fapas
aflatoxin M1	milk, skimmed	spike	0.063	69	22	25	-0.2	Fapas
afltatoxin total	feed, nut based	spike	24.8	33	22	19	1.4	Fapas
afltatoxin total	feed, cereal based	spike	17.1	49	22	28	-0.1	Fapas
DON (deoxynivalenol)	breakfast cereal	spike	587	40	17	21	-0.5	Fapas
DON (deoxynivalenol)	feed (cattle)	incurred + spike	775	32	17	35	1.2	Fapas
Fumonisin B1	maize	incurred	759	66	25	30	6.2***	Fapas
Fumonisin B2	maize	incurred	242	59	25	40	1.3	Fapas
HT2 toxin	oats	spike	113	35	22	26	-0.1	Fapas
HT2 toxin	oats	spike/incurred (43)			22	not available	**	Fapas
ochratoxin A	feed, cattle	spike	41.4	34	22	28	0.9	Fapas
T2 toxin	oats	spike	83.3	41	22	20	-0.9	Fapas
T2 toxin	oats	spike/incurred (11)	65.5	53	22	29	0.9	Fapas
zearelenon (ZON)	feed, cattle, rearing nuts	spike	179	65	21	25	**	Fapas

* Z-scores: between -2 and 2 means adequate performance, between -3 and -2 or 2 and 3 means: questionable performance; < -3 or > 3 means bad performance

** no Z-score provided

*** Z-score of 1.2 after corrective action