National Reference Laboratories RIKILT

Annual report 2017

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RIKILT report 2018.009

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Contents

Preface

RIKILT Wageningen University & Research has been assigned several reference-tasks in the field of safety and quality of food and feed. The execution of these reference-tasks is performed in the context of RIKILT's role as National Reference Laboratory (NRL) in various fields. NRLs are the link between the European Union Reference Laboratories (EURLs) and Official Laboratories (OLs). NRLs and OLs perform analyses on food and feed in the framework of the national official controls. NRLs are a centre of expertise for the OLs as well as the Ministries and the Competent Authority.

In this annual report, RIKILT reports on the execution of its NRL tasks in 2017.

Summary

National Reference Laboratories (NRLs) are part of the system responsible for the control and enforcement of EU food and feed law. RIKILT Wageningen University & Research has been designated as the NRL for twelve subjects. The tasks of a NRL depend on its research fields. This report gives an overview of the activities performed by all of RIKILT's NRLs in 2017. These NRLs are for: dioxins and polychlorinated biphenyls (PCBs), pesticides in products of animal origin, mycotoxins, heavy metals, polycyclic aromatic hydrocarbons (PAHs), marine biotoxins, certain substances and residues thereof as laid down in Directive 96/23/EC, genetically modified organisms (GMOs) in food and feed, animal proteins, feed additives, milk and milk products, and water content of poultry.

This report first gives an overview of relevant legislation and information on the networks of EURLs, NRLs and OLs. For every NRL, a description is then given of all activities performed in the EURL-NRL network such as participation in EURL-NRL workshops, working groups, and proficiency and comparative tests. This is followed by a description of the assistance given to OLs in the form of quality control and/or advice. Finally, the scientific and technical support given to the competent authority is discussed. In some cases, the contact with other NRLs is discussed.

An important NRL task is to stay up to date with current developments within its NRL domain. Every EURL organizes one or two meetings (workshops) every year for that purpose. Participation in these EURL-NRL workshops is mandatory. In 2017, 18 workshops have been attended by RIKILT's NRLs. Additionally, the NRLs have actively participated in EURL working groups to improve analytical methods. To test the analytical capabilities of NRLs, the EURLs organize proficiency tests. Due to EURL proficiency tests sometimes being limited in their scope, the NRLs have also participated in proficiency tests organized by other organizations if thought to be relevant. Most results (Z-scores) in these proficiency tests were good; only a few 'questionable' and a single 'unsatisfactorily' result were reported. Follow-up actions were implemented in those cases. The performance of the OLs has been assured by checking the results of their performance in proficiency tests (organized by other laboratories or the NRL) or by sending assurance-samples. Some OLs have also received technical support with regard to their analyses.

1 Introduction

Food law aims to assure a high level of protection of human life and health and to achieve the free movement of food and feed marketed in the European Union. Food and feed businesses are to ensure that they satisfy the requirements of food law. National authorities are to enforce food law, and monitor and verify that the relevant requirements are fulfilled by food and feed business operators at all stages of production, processing and distribution. The manner in which official controls are carried out is prescribed in European and national rules. In the context of those official controls, samples for testing have to be taken. A large amount of sampling is done in the context of multi-annual national control plans set up by the competent authorities of Member States, in accordance with broad guidelines drawn up at the Union level. To ensure the uniformity of analytical results, requirements have been set for laboratories, sampling, and analytical methods. For this purpose, European Union Reference Laboratories (EURLs) are tasked with the support of National Reference Laboratories (NRLs). Pursuant to EU legislation, every Member State is obligated to designate at least one NRL per EURL. NRLs are, *inter alia*, expected to stay up-to-date with scientific advances within their field and are tasked with the support of those laboratories where official samples are tested – the official laboratories (OLs). RIKILT Wageningen University & Research has been designated as the NRL for 12 subjects by the Ministry of Economic Affairs and the Ministry of Health, Welfare and Sport. These subjects are:

- Dioxins and polychlorinated biphenyls (PCBs)
- Pesticides in products of animal origin
- Mycotoxins
- Heavy metals
- Polycyclic aromatic hydrocarbons (PAHs)
- Marine biotoxins
- Certain substances and residues thereof laid down in Directive 96/23/EC
- Genetically modified organisms (GMOs) in food and feed
- Animal proteins
- Feed additives
- Milk and milk products
- Water content of poultry

The objective of this report is to give an overview of activities performed by RIKILT's NRLs in 2017.

1.1 EU Legislation

In 2017, the new Official Controls Regulation (Regulation (EU) No 2017/625) was officially published. This new Regulation amended and repealed several food safety Regulations. However, most provisions regarding reference laboratories in this Regulation (Article 92-101) only applied from 2018. The only provision that went into effect in 2017 (28 April) is Article 163[1], which amends Regulation (EU) No 652/2014 on the management of expenditure relating to the food chain, animal health and animal welfare. This amendment changed the financial support structure for official controls; specifically on which costs incurred to implement the work programmes are covered. The following overview of applicable legislation therefore contains only few adjustments compared to the 2016 report. A complete overview of this new legislation will be included in the 2018 report.

The most important legislation in the EU on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules is Regulation (EC) No 882/2004. This Regulation mandates that Member States uniformly monitor and verify that at all stages of production, processing and distribution the relevant requirements are fulfilled. In addition to the Official Controls Regulation, more specific legislation may apply to certain parts of the production chain

[1] See Article 167(4) of Regulation (EU) No 2017/625.

or certain subjects. For instance, additional provisions for the official controls of residues of veterinary medicines and banned substances in the production of animals for food production are laid down in Directive 96/23/EC. Moreover, additional provisions have been laid down for residues of plant protection products in Regulation (EC) No 396/2005; for feed additives in Regulation (EC) No 1831/2003; for genetically modified organisms in Regulation (EC) No 1981/2006 (and recommendation 2004/787/EG); and for animal proteins in Regulation (EC) No 999/2001 and Regulation (EC) No 1069/2009. For the official controls on the water content of poultry, additional provisions have also been laid down (Regulation (EC) No 543/2008). Those Official Controls are carried out to ensure the functioning of the Single Market.

1.2 Competent authorities

Member States are to designate competent authorities responsible for official controls. The Netherlands Food and Product Safety Authority (NVWA) is the CA for most products in the Netherlands, but for milk, milk products, and egg and egg products this is the 'Centraal Orgaan voor Kwaliteitsaangelegenheden in de Zuivel' (COKZ). The CA is responsible for designating laboratories where analysis of samples, taken for official control purposes, takes place. In addition, the CA is responsible for making the multi annual national control plan (MANCP) which includes attention to physical controls (sample analysis).

1.3 European Union Reference Laboratories (EURLs)

EURLs are designated by the European Commission. Laboratories have been invited to become an EURL via a tendering procedure. The list of EURLs is laid down in Annex VII of Regulation (EC) No 882/2004. Table 1 shows the EURLs relevant for the NRLs of RIKILT.

Table 1 *List of EURLs relevant for NRLs RIKILT*

EURL	Substances/products
Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg Freiburg Germany	• Dioxins and PCBs in food and feed • Residues of pesticides in food of animal origin and commodities with high fat content
Fødevareinstituttet Danmarks Tekniske Universitet København Denmark	• Residues of pesticides in cereals and feedingstuffs
Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart Fellbach Germany	• Single residue methods for pesticides
The Joint Research Centre of the European Commission Geel Belgium	• Mycotoxins • Heavy metals in food and feed • Polycyclic Aromatic Hydrocarbons (PAH)
Agencia Española de Seguridad Alimentaria (AESA) Vigo Spain	• Marine biotoxins
RIKILT Institute of Food safety, part of Wageningen UR Wageningen The Netherlands	• Stilbenes, stilbene derivatives, and their salts and esters (A1)* • Antithyroid agents (A2) • Steroids (A3) • Resorcylic acid lactones including zeranol (A4) • Sedatives (B2d) • - Mycotoxins in animal products (B3d)

EURL	Substances/products
ANSES – Laboratoire de Fougères France	• Antibacterial substances, including sulphonamides, quinolones (B1) • Dyes (B3e)
Bundesamt für Verbrauchershutz und Lebensmittelsicherheit (BVL) Berlin Germany	• Beta-agonists (A5) • Anthelmintics (B2a) • Anticoccidials, including nitroimidazoles (B2b) • Non-steroidal anti-inflammatory drugs (NSAIDs) (B2e)
Instituto Superiore di Sanità Roma Italy	• Chemical elements in animal products (B3c)
The Joint Research Centre of the European Commission Geel Belgium	• Additives for use in animal nutrition
The Joint Research Centre of the European Commission Ispra Italy	• Genetically modified organisms (GMOs)
Centre wallon de recherches agronomiques (CRA-W) Gembloux Belgium	• Animal proteins in feedingstuffs
ANSES – Laboratoire de sécurité des aliments Maisons-Alfort France	• Milk and milk products
Board of Experts: JRC (IRMM), DG AGRI and the three NRL's**	• Water content poultry meat

* The compound(groups) followed by () are part of the official controls carried out in the context of Directive 96/23/EC.

** The board of experts is not referred to in Annex VII of Regulation (EC) No 882/2004 but in Article 19 and Annex XII of Regulation (EC) No 543/2008.

EURLs designated within the context of Regulation (EC) No 882/2004 are responsible for (Article 32):

a. providing national reference laboratories with details of analytical methods, including reference methods;
b. coordinating application by the national reference laboratories of the methods referred to in (a), in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;
c. coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing national reference laboratories of advances in this field;
d. conducting initial and further training courses for the benefit of staff from national reference laboratories and of experts from developing countries;
e. providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses;
f. collaborating with laboratories responsible for analysing feed and food in third countries.

1.4 National Reference Laboratories

RIKILT is the designated NRL for many chemical contaminants (see under 1 above), GMOs, animal proteins, milk and poultry meat. A working plan describing the tasks for 2017 has been drafted in 2016. In addition, budgets for personnel, and facility and equipment costs have been drawn up.

The working plans for 2017 have been positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of Economic Affairs and the Ministry of Health, Welfare and Sport) and has been presented to the director of the Department Agriculture and Nature Knowledge ('Agro & Natuurkennis' (ANK)) of the Ministry of Economic Affairs. The Ministry has approved of these plans. Working plans are based on NRL tasks as described in various EU legislation.

As laid down in Article 33 of Regulation (EC) No 882/2004, these tasks are:

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 in accordance with Article 11;
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 adopted in accordance with Article 53;
f. be responsible for carrying out other specific duties provided for in accordance with the procedure referred to in Article 62(3), without prejudice to existing additional national duties.

Within the context of Directive 96/23/EC (Article 14), these tasks are:

- coordinating the work of the other national laboratories responsible for residue analysis, in particular by coordinating the standards and methods of analysis for each residue or residue group concerned;
- assisting the competent authority in organizing the plan for monitoring residues;
- periodically organizing comparative tests for each residue or residue group assigned to them;
- ensuring that national laboratories observe the limits laid down;
- disseminating information supplied by Community reference laboratories;
- ensuring that their staff are able to take part in further training courses organized by the Commission or by Commission reference laboratories.

NRL tasks for animal feed (Regulation (EC) No 378/2005), GMOs (Regulation (EC) No 1981/2006) and poultry meat water content (Regulation (EC) No 543/2008) slightly differ from the tasks described above.

In some cases, the NRLs are listed in legislation. RIKILT has been listed as an NRL in: Decision 98/536/EC (residues of veterinary medicine and hormones (Directive 96/23/EC)), Regulation (EC) No 378/2005 (feed additives), Regulation (EC) No 1981/2006 (GMOs), and Regulation (EC) No 543/2008 (water content of poultry meat).

1.5 Official Laboratories

Pursuant to Article 12 of Regulation (EC) No 882/2004, the competent authorities are to designate specific laboratories authorized to perform analyses of samples taken within the context of official controls. These laboratories are termed 'official laboratories' in this Regulation and 'approved laboratories' in Directive 96/23/EC. CAs may only designate laboratories that operate and are assessed and accredited in accordance with the European standards: EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories', and EN ISO/IEC 17011 on 'General requirements for accreditation bodies accrediting conformity assessment bodies'. Of course, these accreditation requirements also apply to NRLs.

1.6 Methods of analysis

The methods of analysis used to test official samples should be (if possible) validated and included in the laboratory's accreditation (Article 11 of Regulation (EC) No 882/2004). For various compounds and products, specific provisions have been laid down in EU legislation concerning sampling and requirements for analytical methods. Table 2 shows an overview of this legislation.

Table 2 *List of documents with requirements for methods of analysis used in the official control*

Act/Document	For contaminant/residues/products
Regulation (EU) No 2017/644	• Dioxins, dioxin-like and non dioxin-like PCBs
SANCO/11945/2015	• Residues of plant protection products (all matrices)
Regulation (EC) No 401/2006	• Mycotoxins in food
Regulation (EC) No 333/2007	• Lead, cadmium, mercury, anorganic tin, 30MCPD, PAHs and melamine in food
Regulation (EC) No 2074/2005	• Marine biotoxins • Some milk parameters
Decision 2002/657/EC	• Residues of veterinary drugs and hormones (Directive 96/23/EC)
Regulation (EC) No 641/2004	• GMO
Regulation (EC) No 619/2011	• Low level presence (LLP) of GMOs in feed
Regulation (EC) No 543/2008	• Water content poultry meat
Regulation (EC) No 273/2008	• Quality parameters milk and milk products
Regulation (EC) No 152/2009	• All parameters in feed (a.o. GMO, animal proteins, feed additives, contaminants)

2 National Reference Laboratory Dioxins and PCBs in food and feed

2.1 Activities within the EURL-NRL network

2.1.1 Participation in EURL-NRL workshops

Two workshops have been organised by the EURL (CVUA, Freiburg): in Prague (Czech Republic) and in Freiburg (Germany). Frans Verstraete (DG Sante) presented notifications in the Rapid Alert System for Food and Feed (RASFF). There were 14 RASFF notifications for dioxins and PCBs, 12 of which were for feed (including apple pomace from Poland, bentonite from Brazil, minerals from Turkey and China, dried bovine rumen (pet food), fatty acids from Ukraine) and 2 for food samples (sheep meat and hemp oil from NL). Verstraete informed the EURL-NRL network that the documents for analytical criteria for food and feed had been updated (Food: Regulation (EU) No 2017/644 of 5 April 2017 (already in force) and feed: Regulation (EU) No 2017/771 of 3 May 2017 (amending Regulation (EC) No 152/2009, already in force)). He also updated the network on the changes to come with the new official controls Regulation on official controls (Regulation (EU) No 2017/625, replacing Regulation (EC) No 882/2004). He updated the network on changes in assignments of EURLs, being the extension of the scope of the EURL for Heavy Metals to Metals and nitrogeneous compounds; extending the scope of PAHs to processing contaminants; and extending the scope of mycotoxins EURL with inclusion of the plant toxins. Furthermore, it is foreseen that the scope of the EURL for dioxins and PCBs in feed and food will be extended to halogenated persistent organic pollutants (POPs) in feed and food. This change will have consequences for the work programme in the coming years, and consequences for NRLs. The COM/EURL/NRL network concluded that the following analytes or groups of analytes should be covered in the work programme for 2018 – 2020:

- Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD)
- Perfluoroalkyl substances (PFAS)
- Tetrabromobisphenol A (TBBPA), brominated phenols
- Short chain chlorinated paraffins (SCCPs), medium chain chlorinated paraffins (MCCPs)

Some of the above substances are included in the POPs listing (Stockholm Convention), or they are candidates to be included. Verstraete also updated the network on the progress regarding the EFSA opinions for PFAS, and on an EFSA statement regarding the health-based guidance values for dioxins and dioxin-like PCBs. Due to the complexity of this issue, the PFASs working group of EFSA will first come forward with an opinion on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) (expected 2018), and then will work on an opinion on other PFAS.

Updates on the progress of the Core Working Groups (CWGs) of the EURL-NRL network were also provided. Details on the CWG activities are provided in paragraph 2.1.2 below. An important aspect of the workshop is the discussion of the performance of laboratories in the proficiency tests (PTs) organised by the EURL. The Czech NRL described the discovery of a PCB contamination case in the Czech Republic. As main sources of the contamination were identified: colours on barriers and walls, concrete treatment, and coatings of silage pits. In addition, colour coatings were analysed for their PCB content. Positive findings were mainly at buildings built or reconstructed in the 1970s and 1980s. An example of the investigation of a farm with elevated levels of PCBs in milk and muscle meat was given. A coat of paint in the stable could be identified as the main source of the PCB contamination. This underlined again that local contamination sources may affect the feed and food safety on individual farms. The Prague workshop was completed with a visit to the laboratories of the NRL of the Czech Republic.

2.1.2 Participation in working groups

The Dutch NRL participated in three core working groups (CWGs). The CWGs on patterns, on determination of CPs and on Measurement Uncertainty in PCDD/F and PCB Analysis. The CWG on

dioxin patterns got together on May 30, 2017 in Prague (State Veterinary Institute), and in November in Freiburg. At these meetings, several software/pattern recognition approaches were discussed that will aid with the recognition of currently known patterns in case of an unidentified pattern at the time of a crisis. The basic principles of these approaches include: metric distance, classification, correlation, principle component analysis or hierarchical cluster analysis. None of these approaches was preferred over another at first. A set of test patterns was analysed with these approaches in order to test their performances. This testing will continue in 2018.

In 2017, the CWG started collecting PCB patterns from several sources. The Dutch NRL has a very active role in this CWG; coordinating the dioxin pattern collection, as well as contributing with a pattern identification approach ('Determinator').

The CWG Chloroparaffins (CWG CPs), initiated in 2016, got together at two meetings (June, Slovakia and 14 November CVUA, Freiburg). The Dutch NRL also participated in this CWG. Several aspects were discussed in this new field of complex and challenging analysis, including sample preparation and correct instrumental quantification (GC-NCI-MS and direct injection-Orbitrap-MS). The availability of suitable internal standards is limited, and it was decided to investigate if the EURL/NRL network can make these available. Issues with contamination of blanks, separation of SCCPs from MCCPs and response factors were also discussed.

In 2017, the first proficiency test (PT) was organised on CPs in coconut oil. The NRL participated in this PT which consisted of five samples (1 naturally contaminated and 4 samples spiked with commercial standard mixtures). For the naturally contaminated sample, no z-scores were calculated. In the other samples, z-scores of total CP/SCCP/MCCP for sample B were 1.2/0.8/-, for sample C -0.5/-/-0.7, for sample D -0.6/-1.3/0.4, and for sample E -0.8/-1.1/-. Because of these promising results the NRL was invited to present their analytical approach in the CWG meeting in November. The CWG on Measurement Uncertainty in PCDD/F and PCB Analysis has finalised their work in 2017, and the CWG, in which the NRL participated, was discharged. The final work of this CWG included the editing and finalisation of the Guidance Document, which was published in 2017.[2] At the CWG meeting in Freiburg, it was decided to start with new CWGs in 2018, being a CWG on brominated flame retardants (BFRs) and a CWG on per- and polyperfluoroalkyl substances (PFAS). The CWG on patterns and the CWG on CPs will continue their work in 2018.

2.1.3 Participation in proficiency and comparative tests

The NRL has participated in all PTs on food and feed organised by the EURL. In addition, the NRL participated in PTs organised by Folkehelsa (Norway) and QUASIMEME. The EURL round one samples were palm fatty acid distillates (PFAD). All z-scores for dioxins and PCBs were satisfactory, except for PCB 123 and 189. Here the problem was that the result of the NRL was reported as a <LOQ value, which was above the actual assigned value. The NRL result was taken as a true measured result, which was an incorrect assessment by the EURL. This phenomenon also occurs with the Folkehelsa PTs, as discussed below.

The second PT round concerned liver samples. In this round, all z-scores of the NRL were good. Participation in the Folkehelsa PT for dioxins and PCBs was successful for nearly all analytes for the matrices sheep meat, cod liver and an analyte solution. The <LOQ issue also led to an elevated z-score for Sum TEQ for sheep meat (z=2.1). Concerning the Folkehelsa sum-PBDE and HBCDD PT in sheep meat and herring, there were 7 observations where the LOQ of the NRL was higher than the assigned value, leading to z-scores >2, similarly as described above. The follow-up for this issue is that the organiser (Folkehelsa) was contacted to discuss this problem. There is no reason to conclude that the NRL's performance on these analytes is not sufficient. In fact, for the QUASIMEME PT on turbot liver and sea bream comprising *individual* PBDEs, satisfactory z-scores were obtained. For HBCDs, the <LOQ value issue played a role as well, but good z-scores were achieved for a-HBCDD in an analyte solution and a herring sample.

[2] https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_document_pcdd-f_pcb_en.pdf

2.2 Assistance to official laboratories

2.2.1 Quality control

In two rounds (spring and autumn), eight dairy fat samples were exchanged with the OL. Two samples of 2016 were repeated again in 2017, to monitor the performance over time, which was good. The results of the OL were good in all cases, although a slight negative bias for CB-126 and the dioxin-TEQ was observed in all cases. This was reported back to the OL. In addition to previous years, the repeatability and reproducibility were evaluated.

2.2.2 Advice

Information from the EURL-NRL network was exchanged with the OL.

2.3 Scientific and technical support to the competent authority

There has been frequent support of the ministries of economic affairs (MinEZ), of health, welfare and sports (VWS), NVWA and RIVM concerning dioxins and PCBs in eel, Chinese mitten crab, and food and feed in general. The current activities on PFASs and CPs were also brought to the attention of these organisations. Support was given on the interpretation reports of analysis of commercial laboratories in specific cases.

2.4 Contacts with other NRL's

The NRL contacted the EURL and the NRL of Belgium during spring 2017 to evaluate a new automatic system for sample clean-up for confirmatory analysis. In addition, there has been frequent contact with other NRLs to discuss topics on dioxin patterns. The outcomes of these discussions and other activities in the EURL-NRL network were presented at the following occasion:

Dioxin 2017 conference
R. Malisch, T. Bernsmann, R. Ceci, G. Diletti, G. Eppe, A. Fernandes, H. Fiedler, J. Hart, R. Hoogenboom, H. Hove, G. Knetsch, L. Leondiadis, A. Lüth, P. Marchand, S. Maszewski, M. Rose, A. Schaechtele, H. Vanderperren, S. van Leeuwen, L. van Raamsdonk, I. Vassiliadou (2017) Interactive data base of PCDD/F and PCB congener patterns to aid identification of contamination sources in feed and food, Dioxin 2017, August 20-25, Vancouver, Canada

Reports
Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry, available through https://ec.europa.eu/food/sites/food/files/safety/docs/animal-feed-guidance_document_pcdd-f_pcb_en.pdf

3 National Reference Laboratory Pesticides in products of animal origin

3.1 Activities within the EURL-NRL network

3.1.1 Participation in EURL-NRL workshops

For pesticides, there are four EURLs, three covering a type of commodity (fruit & vegetables, FV; cereals & feed, CF; products of animal origin & high fat content, AO), and one covering pesticides that are not amenable to multi-residue analysis and need dedicated single residue methods (SRM). In the EURL-NRL network, one workshop is organized every year. In odd years, this is a joint event by all four pesticide EURLs. In even years, separate meetings are held by the individual EURLs.

In 2017, a joint meeting was held on 27-29 September in Freiburg, Germany, which was attended by RIKILT. RIKILT shares the NRL task with NVWA, that also attended the meeting. The purpose of the workshop was to inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, new technologies, issues with certain pesticide/matrix combinations), to present the set up and discuss the outcome of the annual EU proficiency tests, and to present the EURL program and activities for the next year. Furthermore, the bi-annual revision of the "Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed" was presented and discussed.

The items that were presented and discussed at this meeting are briefly summarized below:

- Jan von Kietzell from Health and Food Audits and Analysis (HFAA, formerly the Food Veterinary Office (FVO)) / DG SANTE / European Commission gave a presentation on audits conducted by HFAA. Specific audits were held on pesticide residue controls in organic production. It was noticed that within the EU there was a lack of harmonisation regarding scope and limits of determination, and also on the interpretation and follow-up when pesticides are detected in organic products. The presentation further dealt with an overview of audits (to be) held in non-EU countries and EU countries. For non-EU countries, the priority was on audits in countries with a high non-compliance rate in RASFF and EFSA reports. In EU countries, a new series of audits has been scheduled for 2018-2019, which include designation of NRL, and OLs, follow up on EUPT results, requirements for risk-based and random sampling, and enforcement actions. The EURLs will be involved in the audits.

- Six technical presentations were held. Silja Laufer (EURL-AO) compared various clean-up procedures for GC-MS-based analysis of pesticides in food of animal origin. Susan Hermann (EURL-CF) also addressed clean-up procedures, but for cereal food and feed matrices. Different existing and new adsorption materials were compared. Friederike Habedank (LALLF M-V, Rostock) presented an automated clean-up procedure based on two-dimensional liquid chromatography (2D-LC) combining HILIC and reversed phase columns. Amadeo Fernandez-Alba (EURL-FV) gave a lecture on LC-HRMS (liquid chromatography coupled to high resolution mass spectrometry), which is an emerging technique in routine pesticide residue analysis. Currently, one third of the laboratories participating in the EU screening PT is using this technique, which is beneficial with respect to scope and the ability for retrospective analysis, so it has the potential to detect more pesticides. However, there is still a trade-off with sensitivity and cost of the instrument compared to targeted methods based on LC-MS/MS. Michelangelo Anastassiades (EURL-SRM) gave an update on methods ("QuPPe") for highly polar pesticides that need dedicated extraction and LC-MS/MS conditions for their determination. Attention was paid to fluoracetic acids, and stability of standard solutions. Also the issue of phytogenic CS_2 (marker substance for dithiocarbamates) was addressed. Jens Luetjohann (Galab, Germany) also presented on highly polar (anionic) pesticides (HEPA, ethephon, chlorate, perchlorate, phosphonic acid, phosphoric acid, fosethyl, bromide) using a method based on ion chromatography (IC) coupled to MS/MS.

- Fipronil in eggs. Ralf Lippold (EURL-AO) gave an overview of the fipronil incident that became apparent mid-2017 following residue findings in eggs, and an overview of findings in Belgium, The Netherlands and Germany. Maximum residue limits (MRLs) and processing factors for egg products were presented, as well as a recommendation for monitoring of other acaricides besides fipronil with potential for illegal use. In retrospect, it was found out by the EURL-AO that the egg sample provided for the 2017 EURL-proficiency test (PT), organised in April, did contain fipronil-sulfone (approx. 8 µg/kg). As it was outside the scope of the PT and monitoring programs at that time, laboratories were re-invited in September to perform an additional analysis of the egg sample for residues of fipronil/sulfone. 29 laboratories submitted results. In general, (92%) acceptable results were obtained. The inter-laboratory variability was 14% (robust relative standard deviation) showing that analytical performance is good for this pesticide amongst NRL/OLs in various EU countries.

- Discussion of EURL-proficiency tests (PTs). In total five PTs were organised in 2017 by the various EURLs: multiple pesticides in egg (AO12), oats (CF11), lemon (FV19 and SM09) and specific SRM-pesticides in strawberry purée. In each of the five presentations the setup, preparation of the test materials, and the results were presented. Specifically for products of animal origin: the matrix was egg. A target list was provided with 59 mandatory pesticides and another 25 pesticides to be analysed on a voluntary basis. In total 13 and 5 pesticides, respectively, were present in the material. Levels ranged from 0.026 to 0.31 mg/kg. In total, 104 laboratories (NRLs and OLs, plus several laboratories from third countries) participated. For the mandatory pesticides, the percentage of laboratories that reported that pesticide ranged from 64% to 97%. For the voluntary pesticides, this was substantially lower, ranging from 31-64%. The percentage of laboratories with acceptable performance varied for the different pesticides, from 83-95%. As a measure for the inter-laboratory variability, the robust relative standard deviation was used which varied from 13% to 32%. In most cases, this was around or below the percentage of 25% that is used as a fixed value for expanded measurement uncertainty for enforcement purposes.

- AQC document. A full session was devoted for presentation and discussion of the proposed revision of the revision of the analytical quality control (AQC) document: "Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed" (existing version: SANTE/11945/2015), moderated by Tuija Pihlstrom (National Food Agency, Sweden). Main modifications were on identification criteria when using high resolution mass spectrometry (presented by Hans Mol (RIKILT) and Amadeo Fernandez Alba (EURL-FV, University Almeria, Spain)), a clearer description for assessment of and criteria for linearity (explained by Hans Mol), the requirement to correct for recovery when the recovery is outside the range 80-120%, and an update of the commodity table for feed commodities (Mette Poulsen (EURL-CF, DTU, Denmark)). After discussion, the modifications were adopted. A new version of the AQC document (SANTE/11813/2017) to be used by January 1, 2018 was issued.[3]

- Various EURL matters: the CIRCA BC website where NRLs can find different kinds of information was presented by Ralf Lippold (EURL-AO, CVUA Freiburg, Germany). The next EU-PTs were announced (CF, hay; FV, green beans; AO, milk(powder); SRM, soybean).

3.1.2 Participation in working groups

NRL RIKILT is member of the AQC-working group for the bi-annual revision of the 'Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed'. Proposals for and comments on modification of the document were done. The draft revisions were more thoroughly discussed during two meetings that were attended: 28th June in Madrid and 26th September in Freiburg (preceding the Joint Workshop).

3.1.3 Participation in proficiency and comparative tests

The RIKILT NRL participated in two proficiency tests in the domain of pesticides in products of animal origin. One concerned egg, organised by the EURL in April. In September an 'add-on' to this PT was

[3] https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf

done focusing on fipronil (details see 3.1.1.). The other PT concerned the determination of LC-MS/MS amenable pesticides in liver. For liver and fipronil in egg, the pesticides were correctly quantified (z-scores within ±2). For several other pesticides in egg, a positive bias was observed, resulting in deviating z-scores. Follow up was performed, the cause for the deviating scores was found (error in a calibration solution), and corrective action was taken.

3.2 Assistance to official laboratories

3.2.1 Quality control

In the Netherlands, there are two laboratories involved in official analysis of samples of products of animal origin in general: RIKILT and NVWA. Both are also NRL for this domain. In addition, there is one laboratory performing part of the official analysis of dairy products. In the frame of a quality control program, samples of milk powder were sent to the dairy laboratory for analysis of organochlorine pesticides. Results were reported to and evaluated by RIKILT.

3.2.2 Advice

RIKILT reviewed two reports from proficiency tests on organochlorine pesticides and PCBs organised by Ducares for private laboratories.

3.3 Scientific and technical support to the competent authority

Contributions were provided to the competent authority in the frame of Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products. This concerned the scope of analysis of pesticides in products of animal origin when monitoring in a risk-based context, and in particular acaricides, ectoparasiticides and insecticides that could potentially be misused in poultry.

At the request of the competent authority (NVWA), following the onset of the fipronil incident (eggs, poultry), a quality assurance program was set up for private laboratories in the Netherlands to assess capabilities and comparability of analysis results. Egg samples with incurred residues of fipronil and fipronil-sulfone were prepared and distributed amongst eight laboratories. Feedback was provided. In addition, an audit was performed and a meeting with the laboratories was attended.

3.4 Contacts with other NRL's

Regular ad-hoc contacts took place with the other Dutch NRL on pesticides in animal origin (and other commodities) throughout the year. Furthermore, dissemination and discussion of technical aspects (analysis, legislation, analytical quality control) took place with representatives from EURLs, NRLs, OLs and other pesticide experts during the joint EURL workshop (see 3.1 and symposia (9th Int. Fresenius Conference Pesticide residues in Food, Mainz, 26-27 June 2017; 8th Int. symposium RAFA, Prague, 7-10 November 2017).

4 National Reference Laboratory Mycotoxins in food and feed

4.1 Activities within the EURL-NRL network

4.1.1 Participation in EURL-NRL workshops

In 2017, a workshop was organized by the EURL for mycotoxins in food and feed (JRC, Geel, Belgium) on 17-18th October in Geel, which was attended by the Dutch NRL. The purpose of the workshop was to inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, standardization) and to present the EURL program and activities for the next year. In anticipation of extension of the analyte domain, plant toxins were also discussed during the workshop. Plant toxins are receiving more and more attention, and were also included as a topic in the program because compound- and matrix-wise it is closest to the mycotoxin domain. During this EURL workshop, the transition of the EURL from JRC, Geel to RIKILT, Wageningen University & Research in the Netherlands by January 2018 was also announced.

Below the items presented and discussed during the EURL Mycotoxins workshop are briefly summarized:

- Discussion of EURL-proficiency tests (PTs). In total, four PTs were discussed, two still dating from 2016, and two PTs from 2017. For the results of the Dutch NRL in 2017 see 4.1.3.The first PT concerned multi mycotoxins, both regulated (deoxynivalenol, aflatoxin B1, zearalenone, fumonisins, T2/HT2-toxin) and emerging mycotoxins (enniatins), in oat and corn. In total, 53 laboratories participated. Enniatins were only analysed by approximately 25% of the laboratories. Good performance was achieved by most of the laboratories (92%) for aflatoxin B1. The determination of HT2 was more challenging, 35% of the laboratories had questionable or unacceptable results. The majority of the laboratories used liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Besides LC-MS/MS, LC with fluorescence detection was also used for aflatoxin B1, while LC with UV detection was used by a number of laboratories for deoxynivalenol. The inter-laboratory variability varied for the different mycotoxins. A value for the relative standard deviation (RSD_R) not exceeding 20-25% is desirable. This was achieved for most regulated mycotoxins except T2/HT2. For enniatins, the RSD_R was very high (41-94%) indicating a need for improvement of analytical methods.

The second PT (2016) was dedicated specifically to aflatoxin B1 in two peanut materials. Assigned values were set by the EURL as 2.8 and 3.2 µg/kg. In total, 56 laboratories participated (40 NRLs, 16 OLs). The majority of the laboratories used LC with fluorescence detection, followed by LC-MS/MS. 92% of the participants reported acceptable results.

The third PT (2017) was on deoxynivalenol in four samples of wheat material. Assigned values were set by the EURL as 551, 1556, 4405 and 1160 µg/kg. A fit-for-purpose target relative standard deviation of 22% was used assessment of laboratory performance (calculation of z-scores). In total, 59 laboratories participated (41 NRLs, 18 OLs). The number of laboratories using LC-MS/MS and LC-UV were similar for this PT. The inter-laboratory relative standard deviation (RSD_R) was 15-20%. Acceptable results were reported by 93% of the participants.

The fourth PT (2017) dealt with ergot alkaloids in rye. EU maximum limits for six ergot alkaloids and their epimers are foreseen for the near future, and monitoring of these mycotoxins is recommended by the Commission (Recommendation 2012/154/EU). Assigned values were based on participants' results and ranged from 116-695 µg/kg for the sum of epimer-pairs. In total, 37 laboratories participated (26 NRLs, 11 OLs). Most laboratories used LC-MS/MS; a smaller number of laboratories used LC with fluorescence detection. Both methods provided equivalent results. Within this PT, analytical reference standards were also assessed. Except for ergometrine (possibly due to a solubility issue), the standards from the laboratories matched well with that of the EURL. The inter-laboratory

relative standard deviation (RSD_R) was below 25% with the exception of α-ergocryptine/inine (34%). Based on the sum of the epimers of the individual ergot alkaloids, 91% of the participants reported acceptable results.

- The first day ended with an overview provided by Jörg Stroka on 10 years of activities performed by the EURL, and the achievements made. On behalf of all NRLs, representatives of the Dutch NRL took the opportunity to thank Jörg Stroka and his team for an outstanding job and all their efforts and achievements in the period 2006-2017.

- Frans Verstraete from the European Commission (DG Health & Food Safety) presented an extended update on issues and regulations for mycotoxins and plant toxins. There are challenges ahead to continue to ensure a high level of human health protection, in a regulatory sense; but also with regards to increasing levels in staple crops due to climate change. The issue of modified ('masked') mycotoxins was addressed. These are mycotoxin metabolites (conjugates) that can be bioavailable and contribute to total exposure. In monitoring, these modified mycotoxins are often not included. The highest relative levels of modified mycotoxins, relative to the parent/free form, occur for zearalenone, deoxynivalenol, nivalenol and fumonisins. It is foreseen that group health based guidance values will be established to take the modified forms into account. For zearalenone, many metabolites and modified forms exist, some of which are more potent in their biological effect than the parent substance. Inclusion in monitoring is therefore considered relevant. For ochratoxin A, maximum levels may be set for additional foodstuffs (cocoa/-products, dried ham). The establishment of maximum levels for *Alternaria* toxins is under discussion, but awaits specific toxicity data.

Plant toxins were also addressed. An update on pyrrolizidine alkaloids (PAs) was given. Discussion is ongoing for possible regulatory measures in honey, tea, herbal infusions and food supplements. A set of 17 (or 21) individual PAs will be regulated. New legislation (Regulation (EU) 2017/1237, amending Regulation (EC) no 1881/2006) has been issued for maximum content of hydrogen cyanide (including hydrogen cyanide from cyanogenic glycosides) in apricot kernels (whole, milled) for direct consumption (20 mg/kg). For opium alkaloids, a target level of 10 mg/kg applies for morphine in poppy seeds for the final consumer. Plant toxins that will receive more attention in the near future include glyco-alkaloids (potatoes) and quinolizidine alkaloids (lupine/-products).

The new 'official controls regulation' Regulation (EU) 2017/625 was explained in detail. This Regulation concerns, among others, methods to be used for analysis ('methods cascade'), aspects related to sampling and analysis, and tasks of NRLs and EURLs.

- Martien Spanjer (NVWA, Netherlands) and Hans Mol (RIKILT, Netherlands) gave an update on the progress of establishment of new harmonised methods for mycotoxins in food and mycotoxins/plant toxins in feed, respectively. In total, 20 CEN standards are being drafted. A few standards have been published recently. For most of them, the inter-laboratory validations have been done. These are expected to be published in the course of 2018 and 2019.

- Birgit Poschmaier (Boku, Austria) gave a presentation on the EU project 'MyToolBox' which is a multi-disciplinary project to reduce mycotoxins throughout the food/feed chain.[4]

- New EURL mycotoxins, with extension to plant toxins, in food and feed.
Following the announcement made in 2016 that JRC will discontinue hosting the EURL mycotoxins by December 2017, a call was launched by the Commission for a new EURL. The outcome was that RIKILT Wageningen University & Research, the Netherlands was appointed as new EURL, per 2018. The extension of the domain with plant toxins means that the competent authorities of all member states will be asked to appoint NRLs for plant toxins. On behalf of the new EURL, Hans Mol (RIKILT) presented the draft working program for 2018. Tasks of the EURL (slightly adjusted by new legislation Regulation (EU) 2017/625) were explained, and options for the scope of proficiency tests and plans for method development were discussed. Monique de Nijs introduced Wageningen University & Research,

[4] https://www.mytoolbox.eu/

RIKILT, and the new EURL team to the NRL community and welcomed the delegates for the next EURL meeting in Wageningen 9-10 October 2018.

4.1.2 Participation in working groups

The Dutch NRL chaired the working group on identification criteria for mycotoxins in food and feed. Following a final discussion during the EURL meeting in 2016, this document was published as a SANTE guidance document in March 2017.[5]

4.1.3 Participation in proficiency and comparative tests

In 2017, the Dutch NRL participated in five proficiency test on mycotoxins. Two were organised by the EURL (deoxynivalenol in wheat, and ergot alkaloids in rye, see 4.1.1.). The other proficiency tests concerned aflatoxin B1, ochratoxin A, deoxynivalenol and zearaelonen in a feed material, deoxynivalenol, zearaelone, T2 and HT2, and sum of T2/HT2 in another animal feed material, and ergot alkaloids in rye flour. The laboratory performance was evaluated through z-scores, which indicated adequate quantitative performance by the NRL.
Anticipating the extension of the NRL domain mycotoxins with plant toxins, the NRL also participated in two proficiency test on plant toxins. One was on tropane alkaloids (atropine and scopolamine) in cereal-based baby food, in which these alkaloids were present at around 1 µg/kg. Adequate quantitative performance was achieved. The other proficiency test, organised by the EURL, was on pyrrolizidine alkaloids in honey and tea. At the time of drafting this document, the report on this PT was not yet received.
A comparative test for the determination of the limit of detection (LOD) was done, with the 'Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food' issued by the EURLs of heavy metals, PAHs, mycotoxins, and dioxins in 2016, as starting point.[6]
Four different approaches for LOD were compared for deoxynivalenol and aflatoxin B1 in wheat and various feed ingredients using an LC-MS/MS based method. The outcome was that the LODs depended on many parameters, including matrix, time of measurement (condition of LC-MS), but also on the method used for estimation of the LOD. LODs for the same mycotoxin in the same matrix measured on the same day were found to differ a factor 2.3 to 5.9, depending on which LOD-estimation method was used. Based on this, it was concluded that 'the' LOD of a method does not exist, and that it would be more appropriate to provide an LOD range, taking inter-sample differences and variations over time into account.

4.2 Assistance to official laboratories

4.2.1 Quality control

In the Netherlands, besides by the NRL itself, official samples are analysed by the OL, and, specifically for dairy products, by one additional laboratory that analyses aflatoxin M1. Both the NRL and the OL analyse most samples using multi-methods also covering many other mycotoxins than the regulated ones. Monitoring the performance of the OL by the NRL takes place in the form of reviewing and discussion the results of the proficiency tests in which the OL participates, and their follow up activities if required. Bilateral quality control of the official laboratories was done through exchange of samples. For the dairy laboratory, quality control was done by sending standard solutions and milk samples containing aflatoxin M1. Results were reported to and evaluated by the NRL, and feedback was provided.

[5] https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-ident-mycotoxins.pdf
[6] https://ec.europa.eu/jrc/en/publication/guidance-document-estimation-lod-and-loq-measurements-field-contaminants-feed-and-food

4.2.2 Advice

No specific advice was requested by the official laboratories. There were ad-hoc contacts with the OL in which technical information was exchanged.

4.3 Scientific and technical support to the competent authority

There are regular contacts between the NRL and the competent authority, both through bilateral meetings and national meetings of the *Expert working group on agricultural contaminants* in which, besides the competent authority and the NRL, also the Ministry of Health and the National Institute for Public Health and the Environment (RIVM) participate. In these meetings, input is provided on technical aspects (e.g. feasible limits of quantification for certain emerging toxin/matrix combinations), and plans for explorative surveys on emerging mycotoxins and plant toxins are discussed.

4.4 Contacts with other NRL's

Contacts with other NRLs were through the EURL workshop, through the CEN meetings on mycotoxins in food and mycotoxins/plant toxins in feed, and symposia (8th Int. symposium RAFA, Prague, 7-10 November 2017).

5 National Reference Laboratory Heavy Metals

5.1 Activities within the EURL-NRL network

5.1.1 Participation in EURL-NRL workshops

For heavy metals there are two EURLs, the EURL for Heavy Metals in Feed and Food (IRMM, Geel, Belgium) and the EURL for toxic elements in food form animal origin (ISS, Rome, Italy) respectively in the frame of Regulation (EC) No 882/2004 and Directive 96/23/EC.

The 12th Workshop of the NRL-EURL network on Heavy Metals in Feed and Food was held in Geel on 26 and 27 September. A total of 53 participants attended the event, representing 28 member states plus Iceland, Norway and Switzerland. During this workshop, results were discussed of the PTs organized by EURL-IRMM on herbal supplements and feed for fish. Information about recent developments of legislation and upcoming new issues concerning e.g. new legislation was presented. Furthermore, elemental speciation (iAs) was discussed based on the outcome of PT HM-25 (see 5.1.3). Frans Verstraete (DG SANTE) presented the recent changes in the European legislation for contaminants in food and feed. Finally, Mr. Verstraete stressed the impact of the newly released Official Control Regulation, Regulation (EU) 2017/625 related to (i) the method cascade; and to (ii) the mandate, the designation, the tasks and the responsibilities of EURLs and NRLs. A call for selection and designation of EURLs operating in the areas of metals and nitrogenous compounds in feed and food (MeNiCoFF), processing contaminants and mycotoxins and plant toxins in feed and food has been launched in January 2017. The Danish Technical University (DTU) was designated as the EURL-MeNiCoFF to replace the EURL-HM formerly hosted by the JRC-IRMM in Geel.

Jens Sloth, from DTU, presented the newly designated EURL for metals and nitrogenous compounds in food and feed (EURL-MeNiCoFF), hosted by the Danish Technology University (DTU). A broad variety of modern analytical techniques and the active contribution in several ISO and CEN committees of relevance to the determination of elements in food and feed (especially in the field of speciation) constitute major assets of this EURL. In addition, this experienced proficiency test provider has access to a quality reference materials processing facility. The mandate of this EURL is set in Official Control Regulation (EU) 2017/625.

For the NRL-EURL on toxic elements and heavy metals in food of animal origin, a workshop was held in Rome on 19 and 20 October. During the introduction of the workshop, the EURL-Director stressed the changes related to the tasks of the EURLs, as well as the NRLs, reported in the Regulation (EU) 2017/625. Furthermore, results were discussed on the PTs organized by EURL-ISS on freeze-dried turkey and milk, information and notes on legislation, results interpretation in relation to the maximal limits of metals in food, analytical measurements procedures, and upcoming issues concerning e.g. new legislation were presented and discussed. Furthermore, theoretical exercises were done on the harmonization of compliance assessment for compound and processed food. Finally the EURL plans for 2018 were discussed: there will be a PT on frozen fish in April and a PT on transformed food in July.

In 2017 the EURL for Heavy Metals in Feed and Food and the EURL for toxic elements in food of animal origin organised proficiency tests on the determination of methyl-mercury (meHg), inorganic-arsenic (iAs) and total cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and nickel (Ni) in food and feed. The main objective of this exercise was to evaluate the capabilities of the NRLs in the determination of heavy metals in food (maximum levels in Regulation (EC) No 1881/2006) and feed (maximum levels in Directive 2002/32/EC). During the workshops, results of the PTs were discussed. Furthermore, during the workshop in Rome (EURL ISS), the work programme of 2018 was discussed, and the EURL-NRL network agreed on the PT schedule for the coming year. During the workshop in Geel (EURL IRMM) an announcement was made that IRMM will finish there EURL HM tasks by the end of 2017, and that DTU from Denmark will fulfil the EURL tasks from begin of 2018 (see above).

The NRLs are requested to spread the information received during the EURL/NRL workshops towards the official control laboratories and, more in general, to all laboratories performing analysis of food.

5.1.2 Participation in working groups

There were no working groups on EURL-NRL issues in 2017, related to metals in food and feed, to participate in.

5.1.3 Participation in proficiency and comparative tests

Two PTs were organised by EURL-IRMM (Geel), two by EURL-ISS (Rome). Furthermore, to cover the whole scope of the NRL-task, the NRL participated also in three PTs organized by FAPAS and one by PROOF-ACS:

Table 3 *Participation in proficiency and comparative tests, NRL heavy metals*

Name of PT	Topic
EURL-ISS-26	Cd, Cu, total Hg, Pb in freeze dried meat
EURL-ISS-27	As, Cd, Pb, Ni, As, Cd, Pb in Liquid Infant Formula
EURL-IRMM HM-25	As-inorganic, Hg in feed for fish
EURL-IRMM HM-24	Cd, Pb, As, Hg in St. John's worth
PROOF-P1707-RT	Bromide in broccoli
FAPAS 07281	As-total, Hg-total, Methyl Hg in canned fish
FAPAS 07289	As-total, As-inorganic, Cd, Pb, Hg-total in powdered brown rice
FAPAS 07294	Pb, Hg-total in infant cereal

For the proficiency tests on heavy metals, the participants were requested to perform two or three independent analyses, using the method of their choice for the determination of the elements in food and feed. Results of the proficiency tests have been presented and discussed with the NRLs during the EURL-NRL workshops. Results of the FAPAS PT were reported via FAPAS reports only. Some results were below the limit of quantification, therefore no results were reported in the proficiency test. The results for element speciation on meHg as well as the results on total Cd, Cu, Pb, As, Hg, Ni and Br reported by the Dutch NRL were well within the acceptable z-score limits (z-scope should be between -2 and +2). Results for inorganic arsenic were however unsatisfactory. The z-score for this analysis was above 2; namely 3.2. Reanalysis of the material resulted in satisfactory results.

5.2 Assistance to official laboratories

5.2.1 Quality control

Since 2016, the OL does not have accreditation for the analysis of heavy metals in food and feed from the Dutch accreditation board (RVA). The Dutch NRL has since taken over the measurements, and thus the responsibility for the analysis of the elements in food and feed. The OL works side by side with the NRL, so the information concerning quality control for the analysis of heavy metals in food and feed has been discussed on a daily basis. Both the NRL as well as the OL participated in the PTs organized by the EURL, FAPAS and other PTs, and the z-scores were evaluated by the NRL and OL. Furthermore, for quality assurance, the Dutch has prepared a comparison study based on a the analysis of heavy metals in milk samples (food from animal origin). The samples were handed to the OL and results were discussed during a NRL OL meeting. The Dutch NRL has summarised the results in a small report, which was sent to the competent authority (CA).

OL milk and milk products
Analyses for the CA for milk and milk products are carried out by another OL. Therefore, the quality of this OL was also investigated by the Dutch NRL. This investigation included several test rounds with samples with a known metal content, CRM materials or spiked samples were sent to the OL. The

results of analysis by the OL were discussed with the CA milk and reported. All results of the OL were good, z-scores were within the range of -2 and 2.s.

5.2.2 Advice

Since the merger of the laboratories of the OL and the NRL, advice on analytical measurements, quality and measurements strategies are done on a daily basis. Advising the OL for milk and milk products is on ad hoc basis and depends on the outcome of the comparison test organized two times a year by the NRL. In 2017, the results were good and no specific advice was given.

5.3 Scientific and technical support to the competent authority

In addition to heavy metals in food and feed, the elemental speciation of arsenic and mercury species is an important topic in the EURL-NRL network. For the speciation of selenium and arsenic in feed, chrome, mercury, and arsenic in food, RIKILT has developed several speciation methods in the last years in separate method development projects. These methods are based on HPLC coupled to ICP-MS measurements. In 2017 the NRL together with the OL analysed several rice and rice products, fish and algae samples and the results were reported to the CA NVWA.

5.4 Contacts with other NRL’s

During the EURL workshops, the relationship with other NRL was maintained. Furthermore, via e-mail the Dutch NRL contacted the Danish and German NRLs on speciation. In more detail, the speciation of arsenic was discussed with the Danish NRL and the speciation of selenium with the German NRL representatives.

6 National Reference Laboratory polycyclic aromatic hydrocarbons (PAHs)

6.1 Activities within the EURL-NRL network

6.1.1 Participation in EURL-NRL workshops

In 2017, the Dutch NRL participated in the 12th workshop of the EURL for Polycyclic Aromatic Hydrocarbons (PAHs) held on 19 October 2017 at IRMM in Geel (Belgium). Delegates of the National Reference Laboratories (NRLs) and the Directorate General for Health and Consumers (DG SANCO) were welcomed at the meeting by the EURL director. During the meeting legislation, the broadened scope of the EURL in relation to the EURL/NRL tasks and PT results of 2017 were discussed. Hendrik Emons, Head of the Food and Feed Compliance Unit of the JRC in Geel, updated the participants on the recent JRC developments and announced the newly selected host of the EURL for Processing Contaminants: the National Food Institute at the Danish Technical University (DTU), Denmark.

Lubomir Karasek of the JRC showed in detail the outcome of the PTs organised in 2017 by the EURL-PAH for the determination of PAHs in coconut oil and the determination of acrylamide in potato chips. Gerhard Buttinger of the JRC presented the outcome of the method validation study for the determination of MCPD esters and glycidyl esters (GE) in processed food. The trial involved 10 participants, representing a cross-section of research, private and official control laboratories from 4 EU Member States (Germany, UK, Ireland and The Netherlands), USA and Japan. The selection of participating laboratories was based on the performance in a pre-trial, organised prior to the collaborative trial with the participation of 12 laboratories.
Two NRLs expressed, upon EURL invitation, their readiness to share in presentations to the network their activities, experiences or topics for further discussions. The NRL of the Netherlands, RIKILT, gave a presentation about the current work plans and progress on analysis, covered by the EURL-NRL PAH network. The Greek NRL on PAHs presented the Greek experience concerning the necessity to establish descriptors for the description of the food categories listed in Regulation (EC) No 1881/2006. Frans Verstraete of DG SANTE, presented the recent and future developments and provisions of the European legislation on processing contaminants of food.
The NRLs were requested to spread the information received during the EURL/NRL workshops towards the OLs and, more in general, to all laboratories performing analysis of food.

6.1.2 Participation in working groups

In 2016, a working group was formed in the Netherlands that focused on the difficulties in the analysis of PAH in herbs and food supplements. Members of this working group are delegates of Dutch trade/branch organization for the dried herbs and food supplement producing factories, the OL, a commercial contract laboratory and the NRL.
During 2017, the working group held several meetings in Wageningen. The agendas focussed on the differences in analytical approaches, results and outcome of PAH analysis. In 2017 a PT was organized by the NRL to find out if all participating laboratories have comparable outcomes of analysis. More than 20 laboratories participated in this PT. In February 2018, the working group will discuss the outcome of this PT on the analysis of PAH in herbs and food supplements. The conclusions of the working group will be discussed with the commercial contract laboratories and the new EURL (DTU in Denmark).

6.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL has broadened, besides the PAH now also mineral oils, MCPD, acrylamide and furans are included. The Dutch NRL participated also in PTs for these contaminants. In 2017 the NRL has participated in the PT organized by EURL, FAPAS and DUCARES:

Table 4 *Participation in proficiency and comparative tests, NRL PAH*

Name of PT	Topic
EURL-IRMM	PAHs in coconut oil
EURL-IRMM	Acrylamide in potato chips
FAPAS 0669	PAHs in olive oil
FAPAS 2651	MCPD + GE in vegetable oil
DUCARES 450	Mineral oil in fat (maize oil, coconut oil, papeseed fatty acids)

All results of the reported concentrations in above mentioned PTs gave satisfactory results, except dibenzo[a,l]pyrene in olive oil. The reported value was too low, the z-score was marked as "unsatisfactory performance". To improve the method of analysis, the NRL implemented the use of 13C isotope labelled internal standards for 14 out of 16 PAH congeners of interest. Now the result of the sum of 4 PAH and all other individual compounds were satisfactory results in this sample. Furthermore, in a PT on the analysis of 2-MCPD (FAPAS 2651) a z-score of -2.7 was obtained which was marked as "questionable performance". Actions are planned by the NRL to improve performance, the method for 2-MCPD will be improved, and the LOD/LOQ of this method will be updated.

6.2 Assistance to official laboratories

6.2.1 Quality control

On request of the Dutch CA for milk and milk products (COKZ), the NRL performs analysis for PAHs in milk samples twice a year. There is no OL for PAHs in milk in the Netherlands, therefore a quality control sample program is not necessary.
In 2017, the OL for PAHs in other food products was contacted to discuss their results on PAH analysis which respect to their participation in PT's and new information from the EURL on e.g. EU legislation.

6.2.2 Advice

Advice on PAH analysis, determination of PAH in herbs, LOD and LOQ procedures and other information from EURL were discussed with representatives of OL. The NRL analysed official control samples of milk for the CA COKZ, advice to the CA was given on ad hoc basis.

6.3 Scientific and technical support to the competent authority

Due to differences in analytical results reported by commercial contract laboratories in the Netherlands and results reported by OL and NRL, the CA NVWA has started a working group on this topic in 2016. The objective of the working group is to inform as many (Dutch) laboratories on the do's and don'ts in relation to the analysis of PAH in dried herbs and food supplements. This working group had several meetings in 2017, and as a result of these meetings, RIKILT organized a special PT on PAH in herbs and food supplements. In 2018, the final PT report will be published. Results will also be reported to the branch/trade organization, their contracted laboratories and the EURL.

6.4 Contacts with other NRL's

During the EURL workshops, the relationship with other NRLs were maintained. During the year 2017, the Dutch NRL did have contact with EURL about the analysis of PAH in herbs and food supplements.

7 National Reference Laboratory Marine Biotoxins

7.1 Activities within the EURL-NRL network

7.1.1 Participation in EURL-NRL workshops

The EURL annual meeting was held in October 2017 in Baiona, Spain. At this meeting, the results of various proficiency test (PT) studies and other issues were discussed. Paolo Caricato of DG-SANTE attended this meeting to share the vision of the EU Commission on, among others, Brexit. Due to the decision of the UK to leave the EU, there will be a reassignment in 2019 of the tasks of CEFAS (UK). CEFAS is now the EURL for monitoring bacteriological and viral contamination of bivalve molluscs. The legislation on this reassignment is ready and will be published early 2018. The activities currently carried out by the EURL will be assigned to a number of existing EURLs and one new EURL:

- Virus testing (newly designated EURL): The National Food Agency, Uppsala, Sweden. This laboratory will cover all food commodity / virus combinations not just shellfish.
- *E. coli* testing (existing EURL for *E. coli*): The Department of Veterinary Public Health and Food Safety Unit of Foodborne Zoonoses Istituto Superiore di Sanità, Rome, Italy.
- *Salmonella* testing (existing EURL for *Salmonella*): RIVM National Institute of Public Health and the Environment, Bilthoven, The Netherlands.
- Classification and monitoring activities (existing EURL for Marine Biotoxins): Vigo, Spain.
- Vibrios: no EURL network exists and there are no plans to continue activities.

There will be no change in the existing roles and tasks of the biotoxin NRLs. However, the EURL Marine Biotoxins will get an additional role in relation to classification and monitoring activities. It is likely that these activities will be carried out with a number of EURL-NRL working group members. In the Netherlands, this will mean that there will be a closer collaboration from January 2019 between RIKILT (NRL marine biotoxins) and RIVM (currently the NRL for monitoring bacteriological and viral contamination of bivalve molluscs).

Further, there was discussion on the deregulation of pectenotoxins, a topic already discussed on previous meetings. Deregulation is desirable as EFSA concluded (in 2009) that eating shellfish contaminated with marine biotoxins of the pectenotoxin group at maximum levels permitted in the EU does not pose any health risk. The deregulation, i.e. retraction of legal maximum levels, was proposed to the member states in the DG-SANTE EU working group on live bivalve molluscs in 2017.

Unfortunately one member state opposed and therefore pectenotoxins are still regulated. The NRLs asked DG-SANTE to place the topic on the agenda for the next EU live bivalve mollusc meeting of December 2017 in order to have a 2nd attempt for deregulation. Meanwhile, NRLs will contact their competent authorities (present at the bivalve mollusc meeting) to share their vision on deregulation of pectenotoxins. As for the mouse bioassay (MBA), the EURL informed the NRLs that the legislation is to be changed to make the PSP Lawrence method the reference method in the EU for PSP testing instead of the MBA. Reasoning is that the MBA cannot be a reference method since it is a Type IV method for which CODEX criteria are not defined, since the method has never been fully validated. The change in legislation is expected to be published in early 2018.

Next the minutes and conclusions of the previous EURL-NRL (October 2016, Porto, Portugal) workshop were reviewed. An important issue was the monitoring of tetrodotoxins (TTX) in shellfish. EFSA published its opinion on TTX in March 2017. An acute reference dose of 0.25 µg TTX/kg bw was established by EFSA, resulting in a recommended limit for TTX of 44 µg TTX/kg shellfish. There is no legal maximum limit for TTX in seafood yet. A method of analysis for TTX was distributed to those NRLs that were still lacking a method earlier. In the previous meeting, it was already highlighted that there is a need for TTX concentration data in seafood. Unfortunately the suggestion of the Dutch NRL to perform a survey to collect more TTX concentration data for EFSA was not taken over by DG-SANTE. During the presentations of the various NRLs, it became clear that different member

states have a different approach. The Dutch and Belgian authorities have adopted the EFSA recommended value as a legal limit, while other member states are waiting for a decision of the European Commission. Monitoring the presence of TTX is also approached differently by member states. The English and Irish NRLs are performing 'delayed monitoring', this means that a number of samples sampled in 2016 were analysed in 2017. The French NRL will start in 2018 with a TTX survey. The Italian and Norwegian NRLs performed some monitoring on samples that were sent to these NRL. The Spanish NRL has studied presence of TTX in some areas in the Galician Rias. Only Spain and Italy detected TTX, in Italy a single sample was found to contain 500 µg TTX/kg. In Spain, TTX was detected several times, levels found were below 44 µg/kg. Besides the Netherlands, none of the member states included TTX in their national sanitary monitoring program yet.

Finally, the EURL person responsible for the algae working group (WG) gave an update on the activities of this WG (see participation in working groups). The WG will focus on population trends and representative testing methods, emerging risks (i.e. sampling methods for epibenthic microalgae and bacterioplankton), species identification and thresholds, and triggers.

7.1.2 Participation in working groups

The NRL participated in one working group in 2017; the LC-MS/MS working group. No other working group meeting were organized by the EURL. The LC-MS/MS working group reconvened in Brussels in June for a meeting on TTX in shellfish. Various member states presented their current situation and methodologies for analysis with respect to TTX. The EURL presented their work in method development and single lab validation. It became clear that a single reference method for TTX will not be drafted and internationally validated on short term. Member states that need support for implementation of a method can be assisted by the EURL.

A request for participation in a working group on preparation of a guideline marine bio-toxins was sent to the NRLs by the EURL. This request was initiated by the competent authorities; the Dutch CA indicated that they would appreciate such a guideline. The Dutch NRL is willing to participate in this working group, however no meeting has yet been organized.

The working group on phytoplankton monitoring did not reconvene over the course of 2017. However various email discussions were held on how to sample various water bodies, sampling frequencies, counting methods and data interpretation. On behalf of Dutch NRL, an expert of Wageningen Marine Research (IMARES) represented the Dutch NRL in these discussions. Discussions are currently being finalized. It also became clear from these discussions that it will be impossible to harmonize threshold and trigger values for the various algae as situations per member state seriously differ. Therefore, specific approaches per member state will be needed to ensure food safety. The progress of the working group is slow, this is not only due to the complex character of harmonizing the algal monitoring but also to the various approaches of member states that were applied with success in the past.

7.1.3 Participation in proficiency and comparative tests

The Dutch NRL participated in EURL and Quasimeme PTs for ASP toxins (EURL), PSP toxins (EURL and Quasimeme) and lipophilic marine biotoxins (EURL).
Results of the annual PT on the amnesic shellfish poisoning (ASP) toxin domoic acid in shellfish were discussed during the annual EURL-NRL meeting. Again there was not much discussion on the PT for the ASP group as most laboratories had satisfactory results. The Dutch NRL had z-scores below |2|, which indicates that performance is adequate.

In general in the EURL PSP PT the number of non-satisfactory results are higher compared to the other toxin classes. The Dutch NRL performed adequately in testing for total toxicity in two PT samples: for sample 1 a z-score of 0.81 was obtained and for sample 2 this was 1.06. For a third sample, the z-score obtained (-2.81) obtained was inadequate. For the individual toxins in this sample, only for the toxins dcNeo an unsatisfactory result (z-score 3.18) was obtained and GTX1&4 in this sample was missed. This resulted in the unsatisfactory result for the total toxicity (z-score -2.81) in this sample. Both errors can be related to the poor recovery with the ion exchange-SPE cartridges used. Although the method used was a CEN standardized procedure, differences in SPE cartridge batches might be the reason for the poor

recovery obtained for these toxins. Further research in early 2018 will need to confirm these findings and will lead to corrective measures. Furthermore, the NRL participated for PSP toxins in the Quasimeme PT with the LC-MS/MS method. This method is applied in the routine control program in the Netherlands as screening method. All positive samples analysed with this method were identified as suspect and therefore the performance of the screening method was satisfactory.

In the PT on lipophilic marine bio-toxins most NRLs performed satisfactorily. In total three samples were analysed by LC-MS/MS. The Dutch NRL performed satisfactory for the total toxicity content in all samples (z-scores < |2|). For sample 1, a z-score of -0.6 was obtained for OA group toxins and 0 for the yessotoxin group. For sample 2, a z-score of -0.3 was obtained for the OA group toxins, and -0.7 for the azaspiracid group toxins. For Sample 3, a z-score of -0.4 was obtained for the OA group toxins. For the individual analogues the Dutch NRL obtained good results with the exception of 45OH-YTX, here a questionable results was obtained, -2.2 for sample 1. The cause of this error is difficult to trace, as other toxins in the group present in this sample gave excellent z-scores, YTX -0.8, homoYTX 0 and 45OH-homoYTX -0.4. Calculations performed were checked and no errors were found. A more thorough investigation is not possible as no standard materials, both certified standards and reference materials, are available.

For TTX no PT is organised in 2017, during the EURL NRL network meeting the importance of such a PT was highlighted by the various member states. Therefore the EURL decided to organize a PT for TTX in 2018.

7.2 Assistance to official laboratories

7.2.1 Quality control

Besides the NRL, there is one OL in the Netherlands that performs analyses on marine bio-toxins. In order to perform quality control, the NRL advised the OL to participate in the Quasimeme PT scheme for all available toxin classes, respectively ASP, PSP and lipophilic toxins. In 2017 the NRL evaluated the results of the OL in these PTs of 2016 and 2017.

7.2.2 Advice

The results of the 2016 and 2017 Quasimeme PT assessment of the OL were evaluated by the NRL together with the OL. Based on this evaluation, assistance was given on various aspects. The OL was informed of the activities of the NRL in 2017 during the annual NRL meeting held early 2018.

7.3 Scientific and technical support to the competent authority

Over the course of 2017, the NRL, the CA NVWA and Ministry of Health, Welfare and Sport discussed TTX issues several times. Most issues related to the application of measurement uncertainty and how results should be presented in the sanitary monitoring program. In addition, the NRL advised the CA NVWA to support the deregulation of pectenotoxins (see 7.1.1). The NRL was asked by the CA NVWA in a separate project to evaluate the phytoplankton monitoring program and the current algae threshold and trigger levels. A concept report on these topics has been drafted and presented during the NRL meeting early 2018 which was attended by RIKILT, NVWA, OL, RIVM and WMR (IMARES).

7.4 Contacts with other NRL’s

Various NRLs (of Belgium, Germany, Denmark, UK and Ireland), were contacted with questions about their phytoplankton monitoring program. With some NRLs, the Dutch NRL had more elaborated discussions on phytoplankton threshold limits and actions taken.

8 National Reference Laboratory NRL (96/23/EC (WOT-02-003-001)

8.1 Activities within the EURL-NRL network

8.1.1 Participation in EURL-NRL workshops

The NRL participated in the EURL-Workshop in Berlin, held from the 9th until the 11th of May, which was organised by the German Federal Office of Consumer Protection and Food Safety (BVL). This workshop consisted of a theoretical part and a practical part. In addition to the regular program (such as NRCP evaluations, discussing the results of organized proficiency testing, developments in the field of residues for which BVL is responsible (nitroimidazoles, coccidiostats, NSAID's, beta-Agonists and anthelmintics)), the following topics were give attention to:

- investigations on the influence of hydrolysis on the total amount of (marker) residue and consequences,
- update of the NSAID validation project (in which RIKILT is also involved),
- experiences with HRMS Instruments, and,
- discussion on the revision of Decision 2002/657/EC (in particular the points "decision limit", "detection capability" and "method validation").

Secondly, the NRL participated in the EURL-Workshop in Fougères, held on the 21th and 22th of June. This workshop For the Control of Antimicrobial Residues in Food from Animal origin titled 'Screening Antimicrobial Residues by means of Biological en Physico-Chemical methods and revision of CD 657/2002 in respect to Validation of Screening Methods', was organised by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). This workshop consisted of a theoretical part and a practical part. Some topics of this Workshop were: 'Update on Revision of the 657/2002 for Validation of Screening Methods'; 'Technical Developments for Antimicrobial/Dye Residues in Foodstuffs' and 'Overview of Antibiotic Residues PT program over period 2016-2017'.
Thirdly, the NRL participated the EURL-Workshop in Wageningen, held from the 13th until the 15th of November. This workshop also consisted of a theoretical part and a practical part. Some topics of this workshop were:

- 'Update EU-RL activities including Proficiency testing programme',
- 'Discussion on draft documents revision 2002/657',
- 'NRL open forum with topics like:
- 'Reims for screening analysis';
- 'Ion Mobility Mass Spectrometry';
- 'Dry Blood Spot Analysis for steroid esters';
- 'Thiouracil update on animal experimental work'.

Experts from the NRL in the EU Member states, but also representatives of candidate or third countries, participated in the above mentioned workshops.

Other workshop-related activities were:

- The NRL participated in the Regional Conference on Food Security; 'Let's get to work. Building a food secure future', in Hanoi, Vietnam, March 22-23, 2017, and presented a lecture with the title 'RIKILT food safety and reference institute'.
- The NRL participated in the International Conference on Food Science, organised by the Universidad Techonologica Equinoccial; Quito, Ecuador, November 29-December 1, 2017, and presented a lecture with the title 'Chemical contaminants in beverages-old and new safety issues'.
- During a visit of a Korean delegation, from the 20th until the 24th of March, the NRL presented a lecture entitled 'The role of EU and National Reference Laboratories: an integrated laboratory network for food safety'.
- A lecture for the FDA of Iran was given on the 10th of February with the topic "Antibiotics and other veterinary drugs".

8.1.2 Participation in proficiency and comparative tests

The NRL has participated in several proficiency tests organized by the EURLs and other international proficiency testing organizations and has obtained the following scores, as shown in Table 5 and Figure 1 below.

Table 5 *Overview of proficiency and comparative tests*

Description	Organizing institute	Z-score	Assessment
Chloramphinocol in shrimp, quantitative confirmation	FAPAS	Z-score: 0.3	Sufficient
Corticosteroids in bovine liver, quantitative confirmation	Progetto	Between – 0.57 and 1.12	Sufficient
Antibiotics, coccidiostats in compound feed, (quantitative) confirmation	RIKILT	Between -0.34 and 1.88 or identification of the compound was confirmed	Sufficient
Dyes, chloramphenicol and nitrofurans in shrimp, (quantitative) confirmation	EURL-ANSES	Z-score: 0.3 or identification of the compound was confirmed	Sufficient
Beta-agonists in liver, (quantitative) confirmation	EURL-BVL	Between -1.54 and – 0.24 or identification of the compound was confirmed	Sufficient
Tetracyclines in honey, confirmation	FAPAS	Identity of the compound was confirmed	Sufficient
Quinolones in poultry meat, quantitative confirmation	FAPAS	Between -0.2 and 1.2	Sufficient
Nitrofurans in porcine meat, confirmation	FAPAS	Identity of the compound was confirmed	Sufficient
Chloramphenicol in honey, quantitative confirmation	FAPAS	Z-score: -0.2	Sufficient
Beta-agonists in porcine liver, (quantitative) confirmation	FAPAS	Between -1.2 and -0.7 or identification of the compound was confirmed	Sufficient
Dyes in fish, quantitative confirmation	FAPAS	Between -0.3 and 1.4	Sufficient
Synthetic hormones in bovine urine, quantitative confirmation	FAPAS	Between -0.9 and 0	Sufficient
Quinolones in honey, quantitative confirmation	FAPAS	Between -0.4 and 0.6	Sufficient
Coccidiostats in poultry meat, confirmation	FAPAS	Identification of the compound was confirmed	Sufficient
Avermectines and anthelmentics in sheep liver, confirmation	FAPAS	Identification of the compound was confirmed	Sufficient
Chloramphenicol in egg and rabbit meat, (quantitative) confirmation	Progetto	Between -0.19 and 0.12 or identification of the compound was confirmed	Sufficient
Steroids in bovine urine, quantitative confirmation	Progetto	Between -0.14 and 0.09 or identification of the compound was confirmed	Sufficient
Resorcylic acid lactones in bovine urine, quantitative confirmation	Progetto	-1.33 -2.05	Sufficient Deviate result *)
Beta-agonists in bovine urine, quantitative confirmation	Progetto	Between –1.62 and -0.6	Sufficient
Corticosteroids in bovine urine, quantitative confirmation	Progetto	Between -1.66 and 1.35	Sufficient
Tetracyclines in bovine meat, quantitative confirmation	Progetto	Between -0.11 and 0.18	Sufficient
Sulfonamides in porcine meat, quantitative confirmation	Progetto	1.35	Sufficient
Antibiotics in porcine meat, quantitative screening and confirmation	RIKILT	Between -0.9 and 1.05	Sufficient
Corticosteroids in milk, (quantitative) confirmation	RIKILT	Between -1.91 and -0.75 or identification of the compound was confirmed	Sufficient

Description	Organizing institute	Z-score	Assessment
Antibiotics in fish (trout), quantitative confirmation	RIKILT	Between -0.09 and 1.04	Sufficient
Veterinary drugs in feed (high level), quantitative confirmation	Ducares	Between 1.2 and 1.4 11.3	Sufficient Deviate result *)
Veterinary drugs in feed and milk powder, quantitative confirmation	Ducares	Between -0.9 and 1.6	Sufficient
Coccidiostats in feed, quantitative confirmation	EURL Geel	1.23 3.76 and 4.49	Sufficient Deviate result *)

*) Corrective actions taken in response to the deviate results:

Resorcylic Acid Lactones in bovine urine; quantitative result differs from the assigned value. Corrective action: No corrective action because of the minimal deviation (Z-score is 2.05) of the quantitative result of taleranol. Veterinary drugs in feed (high level);quantitative result differs from the assigned value. Corrective action: a new validated method, originating from the EURL JRC Geel, is currently implemented within RIKILT. This method shall be used for the reanalysis of these PT-samples.

Coccidiostats in feed; ; z-score for Diclazuril was 4.49. Corrective action: after checking the data of the analytical series it becomes clear that the used Internal Standard for diclazuril (methyl-diclazuril) was contaminated with diclazuril. After correction for this the z-score was <2. The z-score for narasin was 3.76, for this compound the PT-sample will be reanalysed with the validated method originating from the EURL JRC Geel.

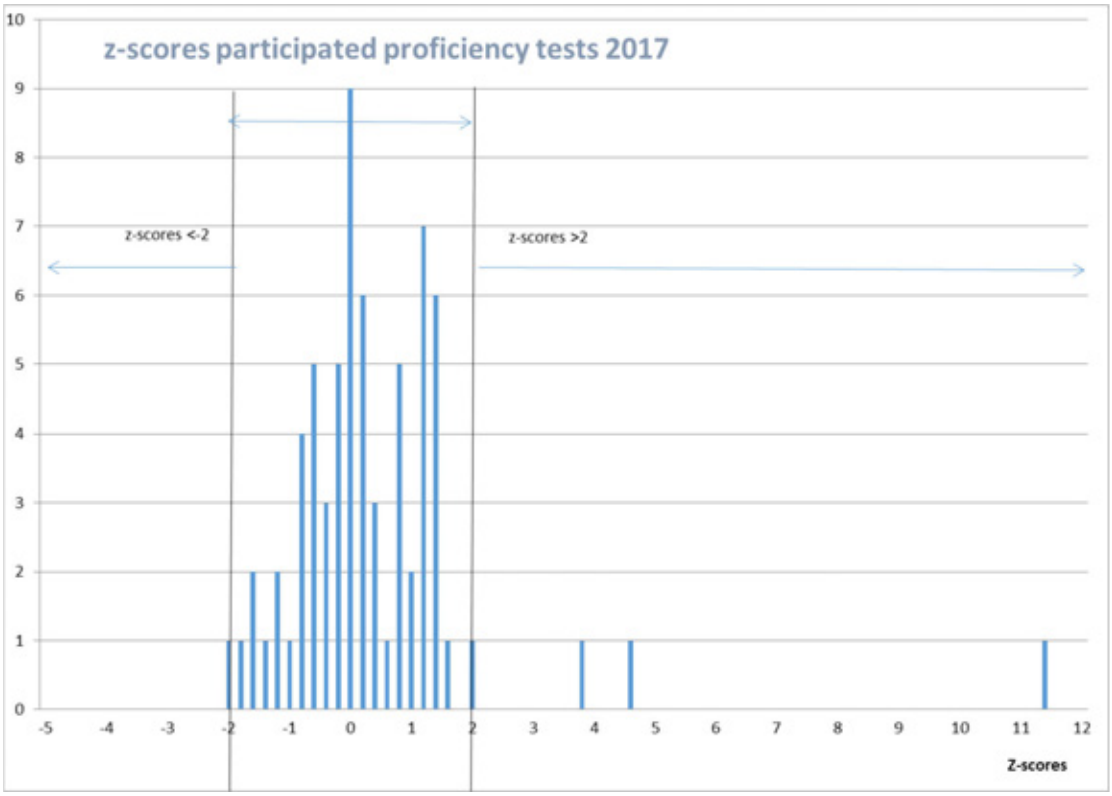

Figure 1 *Overview of the obtained z-scores*

8.2 Assistance to official laboratories

8.2.1 Quality control

Pursuant to the NRL tasks, the OLs are supervised. The NRL assures the analyses of the OL, which are carried out within the framework of the National Sampling Plan, through a third-line control program. The NRL also supports the OL in case of problems, or not corresponding results.

The NRL task (supervise the analysis of the OLs) is only focused on the analyte/matrix combinations from Group A (prohibited) and group B (regulated), mentioned in Directive 96/23/EC. The current control program (third-line control program) includes 39 analyte/matrix combinations. A monthly

evaluation of the outcome of this control program takes place and an annual trend analysis based on those results is performed and reported separately.

Four Technical meeting between the NRL and the OL were held in 2017 to inform one another of developments, discuss analytical issues, and establish corrective actions. This meeting also includes discussing the third-line control program.

8.2.2 Advice

The Q3 meeting (consisting of the NRL, OL and Ducares) was held twice in 2017 (22th of May en 21th of November).
In 2017 the NRL participate in four meetings for the National Plan Residue control workgroup, these were held on the 30 of January, the 19th of June, the 25th of September and on the 18th of December.

A National Expert from the NRL has participated in an FVO audit in South Africa, which was held from the 13th until the 24th of January.
In 2017 the NRL participated in two meetings between RIKILT and the OL with the topic 'the implementation of antibiotic analysis in dairy product which are exported to Russia', these meetings were held on the 14th of March and the 12th of September.

A representative of the NRL participated in a meeting between the NRL and Ducares with the topic: 'catch up on antibiotic residue research', which was held on the 29th of September.

In 2017, contra-expertise was performed five times for the OL. These were for nitrofurazone in feed; clenbuterol in bovine hair; fenoterol in urine originating from goat; semicarbizide in drinking water (for poultry), two times.

The NRL was asked to review the ISO/CD 22186 'Determination of nitrofurazone in milk and milk products'.

9 National Reference Laboratory animal proteins

9.1 Activities within the EURL-NRL network

9.1.1 Participation in EURL-NRL workshops

The annual meeting of the EURL AP and the NRLs was organised in April in Zagreb (Croatia). In one and a half day, a range of topics was addressed. This included the detection of blood and milk, further information on the detection of insects in feed, a technical zero for the ruminant PCR method, the results of the annual proficiency test, a survey on poultry feed, the validation and implementation of the poultry PCR, and the EURL AP working programme.

An important issue for the detection of prohibited ruminant material is the high sensitivity of the PCR method: even traces of ruminant material can be detected. These traces can originate from a low level of carry over between production lines or from traces of milk, which is legally applied. The EURL is working on the development of a "technical zero" below which enforcement is not necessary. It is at least necessary to avoid indication of positive results due to methodological variation. Therefore, this limit is going to be based on a statistical analysis of results of blank samples. Since the purpose of this limit is to avoid unnecessary actions, the term "action limit" will be proposed to the Commission.

The annual proficiency test of 2015 for PCR revealed problems by using different mixes of the necessary chemicals (the 'master mix'). Certain master mixes result in later signals compared to other master mixes. This means that the required type of master mix needs to be defined. RIKILT participated in the survey of different master mixes, and will continue to collect documentation.

New information for the detection of insects was presented. A colouring method for easier recognition of insects particles under a microscope was discussed. The principle of this method, published in a paper in 2017 by the EURL and Italian colleagues, will be elaborated further. Another factor is the concentration of the insect particles by modification of the method for sample preparation. Usually a light and a dense fraction of the sample is prepared using a specific solvent. Some experiments were carried out using a combination with a second solvent in order to concentrate the insect particles in one of the two fractions as precise as possible. This principle of concentration of a specific type of particle was previously developed and applied by RIKILT in the framework of the EU funded project SAFEED-PAP.
A representative of the Commission was present at the meeting and explained new developments in legislation.
The Dutch NRL was present with two experts on light microscopy and PCR. They participated actively in the discussions and in the informal lobby contacts.

9.1.2 Participation in Working groups

In the absence of official working groups, the Dutch NRL seeks cooperation on a bilateral basis.

9.1.3 Participation in proficiency and comparative tests

The final report of the annual proficiency test 2016 was released in March 2017. The PT of 2016 comprised a total of eight samples, all of them to be analysed by microscopy, and five by PCR (ruminant). The Dutch NRL results for the detection of fish by microscopy were all correct, whereas for terrestrial animals the presence of 0.1% of feather meal was not detected. The other seven results were also correct. Nine out of 27 participants did not find the feather material. In two previous versions of the EURL tests, feather meal appeared to be a problem. The difficulty of detecting feather meal needs a follow up. The detection of ruminant material by PCR was correct in all cases where detection was requested (five samples).

The samples for the annual proficiency test 2017 have been sent around in November. This test combined (again) microscopy and PCR analyses. However, in this test the participants were asked to apply the relevant SOPs for their own decision on the correct order of the methods (microscopy and/or PCR). The proficiency test of 2017 comprised a total of eight samples. Results were submitted on time. Preliminary feedback revealed no errors for both microscopy and PCR.

In May 2017, an invitation was sent by the EURL to NRLs for interest in participating in the validation study of the poultry method. This method is targeting chicken and turkey, as most important representatives of poultry. The Dutch NRL has responded positively for participation in this study. The samples were distributed in June 2017, with reporting of results in the same month. Results are not communicated in 2017.

9.2 Assistance to official laboratories

The Netherlands do not maintain a national network of official control laboratories. Therefore, no official activities are performed for this task.

9.3 Scientific and technical support to the competent authority

The Dutch NRL has provided support to the competent authority (NVWA: Netherlands Food and Consumer Product Safety Authority) and to the Ministry of Agriculture, Nature and Food Quality. The aim is to support the constructive position of member state the Netherlands in the process of relaxation of the ban on animal protein. The addressed topics included the application of the technical zero (or reworded to action limit) and the monitoring of poultry material for supporting the anti-cannibalism ban. The coordinator of the NRL together with a representative of the Ministry visited representatives of the Food Hygiene Unit of the EC Health and Food Safety Directorate-General in order to discuss several topics on the future of the animal protein regulations.

A survey was carried out to document the relationship between legislative demands and available monitoring methods. The presence of definitions appeared to be pivotal to this relationship. The survey, its evaluation and recommendations are intended to be published in a scientific journal.

Support to the competent authority was provided whenever appropriate.

9.4 Contacts with other NRLs

The Dutch NRL serves as scientific officer and qq. as board member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are member of this section. During the IAG annual meeting in June, every year, and if necessary during other meetings, exchange of viewpoints and information is stimulated. RIKILT organises the annual IAG proficiency test on animal proteins in feed on behalf of the board, of which the report is published annually. This flow of information is regularly discussed in the meetings and complements the information of the EURL/NRL AP network.

10 NRL Feed Additives and national evaluation of dossiers / advice WOT-02-004-002

10.1 Activities within the EURL–NRL network

10.1.1 Workshop

The annual workshop for Feed Additive Authorisation was organized by the EURL, JRC-Geel, 21-22 November 2017 in Brussels.
A representative from the European Commission informed the EURL – NRL network about the new Official Control Regulation (EU) 2017/625. Among others, the methods used by NRLs in the framework of official control of feed additives have to be accredited by the end of 2019.
Within CEN/TC 327 Animal Feedingstuffs, under a mandate from the European Commission a method is drafted for the identification of the probiotics *Lactobacillus, Enterococcus, Pediococcus, Bacillus* and yeast in feed. The project leader, a representative of Biosafe Biological Safety Solutions Ltd. in Finland, gave an update about the design of the methods and the progress of the project. The methods will be based on Pulsed Field Gel Electrophoresis.
A representative of AGES from Austria informed the consortium about their experiences with the official EU-method (Regulation (EC) No 152/2009) for urea in feed. This method, based on spectrophotometry, proved to be not completely fit-for-purpose for products that contain simple nitrogenous compounds such as feed grade yeast. The consortium was informed that an enzymatic method is available from VDLUFA. RIKILT informed the consortium that work is in progress to develop a method based on LC-MS/MS.
A representative of the EURL presented the status of methods for verification of trace element chelation. Trace elements are authorized separately according to their different forms and the proper use of the different additives requires suitable methods of analysis for their distinction. The method recommended by the EURL for authorized zinc and copper chelates with soya protein hydrolysates is FTIR, combined with multivariate statistics.

10.1.2 Dossier evaluation on request of the EURL

In 2017, the NRL commented on nine initial evaluation reports prepared by the rapporteur laboratory. The evaluation concerned the methods of analysis that were submitted in the dossiers. The advices were described in evaluation reports and included the following additives: technological additives (substances for reduction of the contamination of feed by mycotoxins, acidity regulators), sensory additives, coccidiostats, and nutritional additives (amino acids, trace elements).

10.1.3 Appointment as NRL for Feed Additives Control

Since the start of the EURL/NRL network in 2004, RIKILT Wageningen University & Research has been appointed as NRL in the framework of Feed Additives Authorisation (Regulation (EC) No 1831/2003). In 2017, RIKILT Wageningen University & Research has also been appointed by the Ministry of Agriculture, Nature and Food Quality as the NRL for the official control of additives in feed in the framework of Regulation (EC) No 882/2004. This is important because of the sample analysis performed in the framework of the National Plan for Feed that is coordinated by the Netherlands Food and Consumer Product Safety Authority (NVWA).

10.1.4 Proficiency tests

The EURL has organised two proficiency tests (PTs), viz. for cobalt and coccidiostats. RIKILT, as NRL for Feed Additives Control, participated in both of them.
For cobalt satisfactory results were obtained in the PT with z-scores within the range of -2 and 2. The method that was applied was ICP-MS after microwave digestion.
For coccidiostats the PT contained a blank poultry feed sample spiked with coccidiostats at cross contamination levels. The NRL analysed and confirmed the coccidiostats diclazuril, monensin and narasin correctly. However, the z-scores for diclazuril and narasin were insufficient, resp. 4.49 and 3.76. For monensin, the z-score of 1.23 was sufficient. As corrective action, the validated method from EURL JRC Geel was implemented and validated at the NRL laboratory.

In addition, the NRL participated in two PTs organised by Ducares, the Netherlands. For the first PT which consisted of a cattle feed sample containing coccidiostats, the NRL analysed all components with sufficient z-scores between -2 and 2. The second PT, that was organized after the implementation of the method from EURL JRC Geel, consisted of two samples, a milk powder and a laying mash sample. Some coccidiostats and forbidden drugs were present at cross contamination and low level in these two samples. All compounds were detected and quantified with sufficient z-scores between -2 and 2.

10.2 Scientific and technical assistance to the competent authority

10.2.1 Evaluation of applications for temporary use exemptions of non-authorized feed additives

In 2017, a total of 21 national requests for permission to use substances – which are not authorized at Community level – as additives for experiments for scientific purposes (according to Regulation (EC) No 1831/2003, article 3.2) have been assessed. This assessment concerned the mixing of the additive in feed and possible risks related to cross-contamination to other feeds. The requests concerned among others enzymes, probiotics, vitamins, zootechnical additives and pigments.

Starting from November 2014, the evaluation of GMO-aspects is also performed by the NRL. In 2017, for 24 national requests it was evaluated if it concerned GMOs or additives produced by GMOs. In most cases, it was concluded that the applications indeed concerned additives produced by GMOs. In those cases, it was evaluated if there were specific concerns related to the safety for humans and animals and if the applicant submitted enough information to assess these aspects. In a limited number of applications, supplementary information was requested. In 2017, no application was rejected due to GMO safety aspects.

10.2.2 Other scientific and technical assistance

Among others the Ministry of Agriculture, Nature and Food Quality was advised about the availability and suitability of methods of analysis for the determination of phospholipids in lecithin. Lecithin is authorized as an emulsifier and the recently published Implementing Regulation (EU) 2017/2325 contains a suitable method.

11 National Reference Laboratory for GM food and feed

11.1 Activities within the EURL – NRL network

11.1.1 Participation in EURL-NRL workshops

The Dutch NRL participated in the Steering Committee meeting in February (Ispra, Italy). At this meeting, the 27th Plenary meeting was prepared. It was decided that starting from 2018, the combined Plenary/NRL meeting will be held once a year and extended to 3 days (in October) instead of 2-day meetings twice a year. The Steering Committee will continue to meet twice a year.

The 27th ENGL meeting was attended on 6th and 7th April 2017 in Ispra, Italy. Information was shared from the Steering Committee meeting, the working groups and DG SANTE, e.g. on overviews that have been made on new breeding techniques and synthetic biology. The representative from Finland informed the meeting on the presence of orange petunias in Finland that potentially contained GMO. Apparently, petunias had been transformed with a maize gene in the 80's. This African Sunset petunia contained P-35S, T-nos, kanamycin resistance and a maize gene. As a result of this information at the meeting, later on in 2017 unauthorised GM petunias were detected in several European countries, as well as outside Europe. There was also an interest reported in testing T-shirts for organic cotton (France). It was possible to extract DNA from a T-shirt in sufficient quantity to perform a PCR reaction. In the scientific and technical session there were presentations on e.g. food allergens, digital PCR accreditation, proficiency testing, European FP7 project Decathlon (Esther Kok, RIKILT). There were two breakout sessions with discussions on the new ISO17025 and on the identification of new research topics. Several topics for new research were identified e.g. Next Generation Sequencing for unauthorised GMOs, the setup of a reference sequence database, standardisation and bioinformatics pipeline. There is a need for harmonisation in GMO screening. A workshop in 2018 was proposed.

In June, a training workshop on DNA extraction in Ispra, Italy was attended which was organised by the EURL GMFF with support of LGC (UK). The aim of the workshop was to provide an opportunity to share knowledge and experience on DNA isolation, as DNA isolation is the first and often crucial step for many downstream applications. There were presentations given by EU laboratory representatives (Theo Prins, RIKILT) and by commercial companies. Most of the laboratories encountered similar problems extracting DNA, such as low yield and low DNA purity. These issues were often addressed by the addition of special pre-treatments and clean up steps for specific sample types. Though several official documents provide guidelines for assessment of DNA quality, many labs have in-house procedures to asses DNA quality that are applicable in a routine setting. Most laboratories have experience with a large number of different matrices, but also some specialised labs analyse a limited array of samples for example labs specializing in seed control. Despite this difference, the agreement was that it would be favourable to have a platform available where laboratories can share their experiences with specific sample types. It was also proposed to construct a document that collects the performance requirements mentioned in other official documents. It was proposed to have a similar meeting in 2-3 years. The attendance to this workshop was financed as part of the GMO method validation project WOT-02-004-005 ('Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen').

In September, the 14th NRL and 28th ENGL meetings in Ispra were attended. All NRLs presented their issues, results or training needs at the Tour de Table. Many labs expressed their appreciation of the recent workshop on DNA extraction; it was considered very useful. Some NRLs indicated that a repeat of the workshop in the near future might be useful. Other training needs that were expressed related to the application of (droplet) digital PCR and Next Generation Sequencing, including bioinformatics. In addition, many labs expressed a wish to have a workshop on screening strategies. Other issues that were raised related to the GM Petunia analyses, to the possible detection and identification of (GM) salmon (no official methods are available yet), and the potential detection of GM glowfish. A joint

presentation was given by BVL (Lutz Grohmann) and RIKILT (Theo Prins) to update the ENGL laboratories on the state of the art on GM petunia detection. Lack of 0.1% reference material at AOCS was mentioned. This reference material is needed for quantification at the 0.1% level. There were also presentations on comparative testing results, measurement uncertainty and the DNA extraction workshop. Scientific presentations were given on e.g. CRISPR/Cas9, Japanese GMO regulations, GM Petunia, metabarcoding (Martijn Staats, RIKILT). There were three break-out discussion groups:

1. GM micro-organisms, parameters of a detection method that producers should apply to prove that they can produce the final product without GM-derived DNA was discussed,
2. sample homogenisation,
3. GM animals: DNA extraction methods, and the availability of reference materials and standards for the detection of GM fish (GM Atlantic Salmon, Glofish) were discussed. The GM Atlantic salmon AquAdvantage could be the first food-fish coming to Europa, while the glowfish is an ornamental fish.

At the end of 2017, the digital PCR workshop in Ljubljana, Slovenia, was attended. This was a hands-on training on different dPCR platforms. Some advantages of dPCR are absolute target DNA quantification, removal of PCR bias, high precision, lower sensitivity to inhibition, and detection of rare targets in a complex background. Some disadvantages are that it can be more expensive, that it has a smaller dynamic range, and it is more time consuming. It was announced that in 2018 a digital PCR study will be launched to calculate conversion factors from endogenous and GMO copy numbers to weight/weight percentages for all certified reference materials. ENGL labs with access to digital PCR equipment can participate in this study. The attendance to this training was financed as part of the GMO method validation project WOT-02-004-005 ('Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen').

11.1.2 Participation in working groups

The Dutch NRL continued participation in the Working Group 'Update of the Method Verification', to finalize the document that gives guidance how to verify qualitative screening methods and quantitative GMO detection methods. The Working Group was financed as part of the GMO method validation project WOT-02-004-005 (Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen). The final document was submitted to the Steering Committee.

The Dutch NRL also participates in the WG on ENGL Procedures. The goal of this Working Group is to review the ENGL procedures and where needed propose modifications where needed. The focus is on the procedure to set up ENGL working groups, to come to final ENGL reports and to determine the extent of participation of external parties in ENGL meetings.

11.1.3 Participation in proficiency and comparative tests

Participation in the two proficiency tests organized by the EURL GMFF each year is mandatory for the NRL 882/2004 laboratories. In these proficiency tests, food and feed samples are tested for GMOs and detected GMOs are quantified. The tests contain unprocessed samples as well as more difficult processed matrices. The 2017 proficiency tests included detection and quantification of several GMOs in soya milk (44406, CV127, MON87708 soybean events) and maize flour (MON810, NK603, VCO-1981 maize events). The second proficiency test included ground chicken feed (MON40-3-2 soybean) and soybean flour (MON40-3-2 soya). RIKILT obtained good Z-scores for all quantifications.

11.2 Assistance to official laboratories

11.2.1 Quality control

Both the NRL and the OL participated in the same two comparative testing rounds organised by the EURL-GMFF. The data and the results of the comparative tests were discussed in a joint NRL-OL meeting.

11.2.2 Advice

In 2017, the NRL discussed DNA extraction methods and use of multiple DNA dilutions in quantification in a joined NRL-OL meeting on proficiency test results. The OL also participated in the DNA extraction workshop and the dPCR workshop mentioned in paragraph 1.1.1.

11.3 Scientific and technical support to the competent authority

In 2017 the NRL advised the competent authority with regard to a more risk-based sampling strategy for the Dutch GMO monitoring program. It was discussed that the GMonitor module, developed by RIKILT in 2015, would be used to determine the samples for the GMO analyses in feed in 2018. This module uses available data on the areas of growth of GMOs that have or have not been approved for the European market, to determine the country-crop combinations that are most likely to contain EU-unauthorised GMOs. In addition, information was shared on the EUginius database[7], developed by RIKILT and BVL (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit) and new techniques like Next Generation Sequencing.

11.4 Contacts with other NRLs

Contact with other NRLs in Europe took place during the ENGL Plenary Meetings and the NRL meeting and the DNA extraction workshop in Ispra, and the digital PCR workshop in Ljubljana. In 2016, RIKILT became the NRL GM food and feed for Ireland. In 2017, several web meetings were held and RIKILT visited DAFM in Celbridge in March. The NRL activities for Ireland are financed by Ireland in a separate project.

[7] http://www.euginius.eu/euginius/pages/home.jsf

12 National Reference Laboratory for milk and milk products

12.1 Activities within the EURL–NRL network

12.1.1 Participation in EURL-NRL workshops

The NRL participated in the annual EURL workshop, held on 1-2 June 2017 in Paris (Maisons-Alfort). This annual workshop dealt with all sub-topics for this EURL/NRL domain, being hygiene of raw milk (total flora and somatic cell count) and heat tracers (alkaline phosphatase). At the start of the workshop, the EURL surprised all NRLs present that the EURL would close as of 31-12-2017. A DG SANCO representative explained that the closure of the EURL MMP was proposed by France, claiming that (i) milk quality has improved since the set-up of the EURL in 1992 and is among the safest foods now, (ii) the current scope of the EURL is more dedicated to milk quality than milk safety, (iii) only few official controls are undertaken in Europe on the 3 parameters in the current EURL scope, and (iv) that priority should be given to a new EURL on plant health. DG SANCO followed the French proposal, and EURL MMP is currently delisted as EURL in Regulation (EC) No 882/2004. DG SANCO stressed that member states can maintain their NRLs.

Most NRLs at the workshop expressed that they regret the decision, as there are a number of issues that could still use EURL guidance, as well as that the harmonised state of analysis will gradually be lost, leading to different analytical performance in different European member states.

Following the decision, the EURL was dedicated to finish all running projects before the end of the year, as described per analyte below.

Hygiene of raw milk, total flora
The total flora represents the number of microorganisms present in a certain product. Regulation (EC) No 853/2004 lays down limits for this number, which is $<1*10^5$ colony forming units (CFU) for raw cow's milk, for example. The EU limits are based on reference methods to determine the number of bacteria present, but most EU member states, including the Netherlands, employ routine methods to determine the number in official control samples. During the workshop, the EURL presented that a European conversion line between reference and routine methods is feasible. However, such a conversion factor should be maintained, and without an EURL there does not seem to be a suitable neutral body to perform this task, and the general idea is that this harmonised conversion will not be introduced. The development of guidelines to establish (national) conversion factors, including a sound statistical approach for this, will continue in the ISO/IDF framework.

Hygiene of raw milk, somatic cell count
Somatic cell count (SCC) is the determination of somatic cells in milk, which are a measure of udder health and (absence of) mastitis. For this parameter, also criteria are laid down in Regulation (EC) No 853/2004. The EURL announced that a document specifying critical points during the analysis of SCC and ways to deal with them appropriately would be finalised and shared with the NRLs in 2017. A photo-database with clear descriptions of somatic cells appearance and how to count the different shapes would be prepared (by EURL, DE- and NL-NRL) and shared with all NRLs in 2017. Both topics were requested by the NL-NRL several years ago for better harmonised performance throughout Europe. The Joint Research Centre presented work on a certified reference material for SCC, which is scheduled to be available in 2019. The requesting party (EURL) will not be able to support and lead the introduction of this material to the European practice.

Heat tracers (ALP)
Alkaline phosphatase (ALP) is a native milk enzyme, which activity as such is not particularly important for human consumption, but its thermal degradation curve coincides with that of a proper pasteurisation treatment. Therefore, the determination of the activity of this enzyme in milk and milk products gives information if pasteurisation was performed correctly, and a limit of 350mU/l for cow's milk is present in EU legislation. The EURL attempted to set a limit for ALP in cheeses, and carried out a substantial survey in the past years, but due to exceptional values in a few specific cheeses and the additional burden that a limit could introduce for a few specific cheese producers, the proposal was rejected by the Member states, and this attempt is now abandoned. The EURL presented ongoing work for a multi-well method for determination of ALP which is faster and 'open source', in contrast with the current proprietary reference method. This new method seems to work well, but needs further testing. The EURL will not be able to complete this work, but a Swiss laboratory intends to lead this work further. Reference material for ALP will not be developed further.

12.1.2 Participation in working groups and communication with EURL

The Dutch NRL participated in a working group (EURL, NL, DE) to establish a reference photo-library annex counting guide for somatic cell count, supplying several photos of cells from routine samples, and discussing guidelines to judge the number of cells especially in "difficult" cases. The NRL also commented actively on the draft critical points document (slide preparation and counting) prepared by a working group consisting of 8 NRLs and the EURL. The NRL answered to all EURL enquiries sent in 2017, with help from National parties, on the following topics:
- The national situation in transport and storage of official milk samples for total flora and SCC
- The national position on adopting the EU conversion factor for total flora

12.1.3 Participation in proficiency and comparative tests

The NRL participated in the following EURL proficiency tests:
- PT on ALP in cow's milk (September 2017, 3 samples)
- PT on SCC in cow's milk (September 2017, 3 samples)

In addition to the EURL PTs, the NRL also participated in a number of other proficiency tests:
- PT on somatic cell count (stabilised milk): ALP (Switzerland) (January, May, September, 4 samples each)
- PT on somatic cell count (raw milk): Cecalait (France) (March, June, Sept, Dec, 10 samples each)
- PT on phosphatase (stabilised milk): LGC (UK) (January, May, November, 2 samples each)

In 2017, the EURL reported the results on PTs of the EURL:
- PT on ALP in cheese (2016), the NRL scored satisfactory for 2 samples, but unsatisfactory for 1 cheese sample (z-score 3.6). This has been reviewed, and is believed to be a technical failure which was solved by replacement of a key part of the fluorophos
- PT on ALP in milk (2017), the NRL scored satisfactory again for all samples (z-scores between 0 and 2.6)
- PT on total flora in milk (2016), the NRL scored good for all samples (z-scores between 1.0 and 1.4)
- PT on somatic cells in milk (2017), the NRL scored good for two samples (z-scores 0.5 and 0.6), the third sample was a decoy sample introduced by the EURL and was not evaluated

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2017, the NRL provided assistance to the Official Laboratory by providing results obtained by the reference method for SCC on the internal reference material prepared by the OL. For ALP, two times 10 comparative samples were prepared by the NRL to the OL. For total flora, four series of samples were analysed simultaneously for level checks.

12.2.2 Advisory tasks

On October 2nd, the NRL organised a practical training on somatic cell counting for technicians of the official laboratories. The aim was to review and harmonise the practical details of sample preparation and counting, ensuring comparability of results. Technicians from both OL and NRL were present, along with a few team leaders. The training started with a theoretical introduction, including the recently released EURL guidance documents, and was followed by the preparation of a few microscopy slides from routine milk samples, where lively discussion on the details of the procedure took place. Both the Newman and the ethidium bromide staining were used. The day was completed with joint counting of slides (both staining methods) prepared earlier, focussing on the more difficult cases, thus covering all topics to reach the aim.

13 National Reference Laboratory moisture in poultry meat (WOT-02-005-002)

13.1 Activities within the EURL-NRL network

13.1.1 Participation in EURL-NRL workshops

Brussels
The first expert meeting took place on March 29th 2017 in Brussels. This meeting is the main meeting next to the fall meeting held in one of the member states. The meeting was opened by Alexander Bernreuther. The agenda was approved with the addition of a new topic under AOB on the proposal of German NRL: 'Appearance of a new type of poultrymeat marketed as glazed chicken legs'.

Presentation 2016 Annual report presented to the CMO Committee in December 2016
Alexander Bernreuther (DG JRC) showed his presentation previously given at the CMO Committee – sector animal products in December 2016. This presentation was based on the JRC Technical Report entitled "2016 Annual Report of the Board of Experts in Monitoring Water Content in Poultrymeat following Regulation (EC) No 543/2008".

Short report about the Special Expert Group Meeting 2016 in Turku, FI Status of 'Study on the impact of sample homogenisation on the water content in poultry meat'
Tiina Ritvanen from the Finnish Food Safety Authority - Evira (Finnish NRL) presented an overview of the activities at the Special Expert Group Meeting 'Water monitoring in poultrymeat', held on 13-14 October 2016 in Turku, Finland.

Overview on control data by NRLs 2015 and ISAMM reporting issues
In accordance with Articles 16, 18 and 20 of Commission Regulation (EC) No 543/2008, the NRLs are requested to provide the results of regular checks on water content in poultrymeat to the Commission. The overview on the control data obtained by the NRLs for 2015 was presented by Alexander Bernreuther. The electronic version of the report entitled "2016 Annual Report of the Board of Experts in Monitoring Water Content in Poultrymeat following Regulation (EC) No 543/2008" was provided to all participants. Further, Alexander Bernreuther proposed some guidelines and modifications on the DG AGRI reporting system for the annual control data, ISAMM. This list has been distributed to the NRLs for feedback.

Study on state of play of processing technologies and the absorption of water in poultry meat
Kai-Uwe Sprenger (DG-AGRI) gave an introduction, putting the study in the context of the review of the marketing standards for poultry meat. It was foreseen to review a number of marketing standards and align it to the CMO Regulation (1308/2013) and the Treaty of Lisbon. As this was a complex legal project, immediate amendments to the poultry marketing standards were not foreseen. Moreover, the Commission expected the National Reference Laboratories to give feedback on the results of the present study. Selvarani Elahi (LGC, UK) presented details on the study set-up, difficulties encountered during the study and results of the statistical data evaluation. Further details can be found in the final report of the study.[8]

Study on the impact of sample homogenisation on the water content in poultry meat
Yannick Weesepoel (RIKILT, NL) presented the re-evaluated results of a proficiency test (with 3 different reference materials provided by RIKILT; evaluation according to ISO 13528), as well as the

[8] https://ec.europa.eu/agriculture/external-studies/2016-water-in-poultrymeat_en

results of a "homogenisation study" (= collaborative trial; evaluation according to ISO 5725-2). As it was the very first time that within this expert group such studies were organised.

AOB: Appearance of a new type of poultrymeat marketed as glazed chicken legs
Gisela Hahn (German NRL) presented a recent case of a sample marketed as glazed chicken legs. She provided also photos from the samples, as well as from the label. Although, the samples were labelled "Eisglasiert" ("glazed"), no net weight or drained weight was stated on the label. As this was the first case for the German NRL, she was interested whether other experts made similar experiences and how they would evaluate such a sample from the legal point of view (food law). Expert from Ireland, from Latvia, Slovakia had similar experiences, but only with fish/seafood.

Ploiești,Romania
The fall meeting of experts was held in Romania on 10th and 11th October 2017. Two excursions were planned on 10th October. The first one was a visit to a slaughterhouse from Agrisol in Boldesti-Scaieni, Prahova and the second one was a visit of a poultry rearing farm from Agrisol close to Boldesti-Scaieni, Prahova. During the visit to the slaughterhouse, it was interesting to note that air chilling with moistening was applied. The Expert group meeting was held on the 11th October. The following topics were discussed: after two presentations held by Georgeta Briciu and Cristina Dumitrescu about the Romanian National Sanitary Veterinary and Food Safety Authority and the Structure and tasks of the Romanian Institute for Hygiene and Veterinary Public Health, Alexander Bernreuther (JRC-Geel) presented the first assessment of the 2016 control data.

Evaluation and interpretation of national control data from 2016 by Alexander Bernreuther (JRC-Geel)
A detailed evaluation will be included in the 2017 Annual Report, which will be distributed among the NRLs at the beginning of 2018, as well as at the next Annual Expert Group Meeting in Brussels in March 2018. Among the results, presented by Alexander Bernreuther, it is worth to mention the following findings:

- Compared to 2015, there was a slight increase of the application of air spray chilling for carcases, while the use of immersion chilling decreased. The number of data sets indicating air chilling and "chilling method unknown" remained stable (12% and 4%, respectively).
- For poultry cuts, the trend of reporting "chilling method unknown" further increased in 2016. This is mainly due to imported chicken meat from non-EU countries, but also due to the fact that labelling of chilling methods is not mandatory. Also for cuts, the number of data sets using immersion chilling decreased. It also decreased for air chilling, while it remained stable for air spray chilling.
- About 25% of all data sets for chicken cuts were over-the-limit, more than in 2015 (about 21%). Regarding turkey; about 7% of the data sets for turkey cuts were over the limit, while in 2015 they accounted only for 3%. But one has to keep in mind that when splitting the data into EU and non-EU, about 21% of the EU cuts were over the limit, while about 41% of the non-EU cuts exceeded the limits.
- In more than 62% of the over-the-limit cases for EU samples, the limit was only slightly exceeded (< 0.10), while approx. 50% of the non-EU samples exceeded the W/P limit by more than 0.10. Still, there is a rather constant percentage of cases being largely over the limit (> 0.20) over the last three years for all reported cuts.

Proposals for modification and guidelines for use of ISAMM
In his second presentation, Alexander Bernreuther summarised the status of the proposed modifications of the ISAMM reporting tool for national control data as well as the user's guidelines. A final draft was distributed before the meeting in Romania. The major issues were discussed and NRLs were asked for last comments. The finalised version of ISAMM modifications and guidelines will be distributed to the NRLs for a last verification.

Final reporting on the evaluation of the study on sample homogenisation
Yannick Weesepoel (RIKILT, NL) presented the re-evaluated results of a proficiency test (three reference samples provided by RIKILT; evaluation according to ISO 13528) as well as of the homogenisation study (a collaborative trial; evaluation according to ISO 5725-2). The final report was

delivered to the participants prior to the EURL expert meeting in March 2018. Further ideas on future proficiency tests and a follow-up of the homogenisation study were presented.

13.1.2 Participation in proficiency and comparative tests

There were no proficiency tests held for moisture in poultry meat in 2017.

13.2 Assistance to official laboratories till here

13.2.1 Quality control

The Dutch NRL has organised two quality control rounds (May 2017 and December 2017) in order to ensure that the OL and the NRL obtain statistically similar results for moisture and protein analysis in poultry meat. The quality controls were comprised of two different steps:

- Inter laboratory check of the moisture and protein analysis (homogenised reference samples of chicken fillet meat and chicken legs).
- Inter laboratory check of the influence of homogenisation on the obtained moisture and protein results (intact chicken fillet samples and chicken leg samples).

13.2.2 Advice

The results of both rounds resulted in the observations that the quality of the moisture and protein analysis of the reference samples were acceptably reproducible on an inter laboratory basis. In addition, the analysis of intact chicken fillet samples were within inter laboratory specifications. However, for the chicken legs (bone containing samples), in many cases the limits of inter laboratory reproducibility were exceeded in both quality control rounds. Common causes for reproducibility issues on poultry cuts which contain bones; are unavoidable variation in sample material and differences in homogenisation practises.

13.3 Scientific and technical support to the competent authority

Next to the common scientific and technical support to the competent authorities, there were no special events where the NRL experts were consulted.

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RIKILT report 2018.009

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Report 2018.009

The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 5,000 employees and 10,000 students, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.