National Reference Laboratories WFSR

Annual report 2018

M.Y. Noordam, E. Silletti, A. Alewijn, I.M.J. Scholtens, J. de Jong, L. van Raamsdonk, J.J.P. Lasaroms, A. Gerssen, M.K. van der Lee, J.G.J. Mol and S.P.J. van Leeuwen

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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 2018.

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 2018. 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 organises 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 organise proficiency tests. Due to EURL proficiency tests sometimes being limited in their scope, the NRLs have also participated in proficiency tests organised by other organizations if thought to be relevant. Most results (z-scores) in these proficiency tests were good; only a few 'questionable' and a few '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 (organised 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

Coordinator: Maryvon Noordam

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 have to comply with the requirements of food law; the competent authorities (CAs) of Member States 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, official samples for analytical analyses are taken. A large amount of sampling is done in the context of multi-annual national control plans set up by the CAs as required by EU legislation. 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 to contribute to the improvement and harmonisation of methods of analysis and to support National Reference Laboratories (NRLs). 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 officially re-designated as the NRL for 12 subjects by the Ministry of Agriculture, Nature and Food Quality (LNV) and by the Ministry of Health, Welfare and Sport (Medical Care) (VWS) in April 2018. These subjects are:

- Dioxins and polychlorinated biphenyls (PCBs) (as of March 1, 2018: Halogenated persistent organic pollutants in feed and food)
- Pesticides in products of animal origin and commodities with a high fat content
- Mycotoxins (as of March 1, 2018: Mycotoxins and plant toxins in feed and food)
- Heavy metals (as of March 1, 2018: Metals and nitrogenous substances in feed and food)
- Polycyclic aromatic hydrocarbons (PAHs) (as of March 1, 2018: Processing contaminants)
- Marine biotoxins
- Residues of veterinary medicines and contaminants in food of animal origin (Directive 96/23/EC)
- Animal proteins in feeding stuffs
- Additives for use in animal nutrition
- Genetically modified organisms (GMOs) in food and feed
- Milk and milk products
- Water content of poultry

For 'Milk and milk projects' the EURL was deemed not to be required anymore as of January 1, 2019. The ministries decided however to keep a Dutch NRL for 'Milk and milk products'.
The objective of this report is to give an overview of activities performed by RIKILT's NRLs in 2018.

1.1 EU Legislation

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 this Regulation, more specific legislation applies to certain parts of the production chain 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 been laid down (Regulation (EC) No 543/2008). The controls on water content of poultry are carried out to ensure the functioning of the Single Market.
As of December 14, 2019 Regulation (EC) No 882/2004 (and Directive 96/23/EC) will be repealed and replaced by Regulation (EU) 2017/625, the new Official Control Regulation (OCR). However for the EURLs and NRLs articles in the new OCR became applicable as of April 29, 2018 (and related articles in Regulation (EC) No 882/2004 were repealed). The scope of the OCR is broader than that of Regulation (EC) No. 882/2004, among others, official control on plant health and plant protection products are now also included.

1.1.1 Competent authorities

Member States are to designate competent authorities responsible for official controls, this is also a requirement in the new OCR. In The Netherlands the ministry of LNV is the designated CA, this ministry mandated the Food and Product Safety Authority (NVWA) to perform the tasks of a CA. The CA is responsible for designating laboratories for the analysis of official samples, samples taken for official control purposes. In addition, the CA is responsible for making the multi annual national control plan (MANCP) which includes physical checks (sample analysis) in the different food and feed supply chains.

1.1.2 European Union Reference Laboratories (EURLs)

EURLs are designated by the European Commission. Laboratories have been invited to become a EURL via a tendering procedure. The list of EURLs is still laid down in Annex VII of Regulation (EC) No 882/2004. Table 1.1 shows the EURLs relevant for the NRLs of RIKILT.

Table 1.1 *List of EURLs relevant for NRLs RIKILT*

Substances/product group	EURL
Dioxins and PCBs in food and feed (*scope extended March 1, 2018: halogenated persistent organic pollutants (POPs) in food and feed)*	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg, Germany
Residues of pesticides in food of animal origin and commodities with high fat content	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg, Germany
Mycotoxins (*until March 1, 2018*)	The Joint Research Centre of the European Commission, Geel, Belgium (*until March 1, 2018*)
Mycotoxins and plant toxins in feed and food (*as of March 1, 2018*)	RIKILT Wageningen University & Research, Wageningen, The Netherlands (*as of March 1, 2018*)
Heavy metals in food and feed (*until March 1, 2018*)	The Joint Research Centre of the European Commission, Geel, Belgium (*until March 1, 2018*)
Metals and nitrogenous compounds in feed and food (*as of March 1, 2018*)	National Food Institute, Technical Institute of Denmark, Copenhagen, Denmark (*as of March 1, 2018*)
Polycyclic Aromatic Hydrocarbons (PAHs) (*until March 1, 2018*)	The Joint Research Centre of the European Commission, Geel, Belgium (*until March 1, 2018*)
Processing contaminants ((including PAHs) (*as of March 1, 2018*)	National Food Institute, Technical Institute of Denmark, Copenhagen, Denmark as of March 1, 2018)
Marine biotoxins	Agencia Española de Seguridad Alimentaria (AESA), Vigo, Spain
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)	RIKILT Wageningen University & Research, Wageningen, The Netherlands

Substances/product group	EURL
Antibacterial substances, including sulphonamides, quinolones (B1) Dyes (B3e)	ANSES – Laboratoire de Fougères, France
Beta-agonists (A5) Anthelmintics (B2a) Anticoccidials, including nitroimidazoles (B2b) Non-steroidal anti-inflammatory drugs (NSAIDs) (B2e)	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL), Berlin, Germany
Chemical elements in animal products (B3c) ((*until January 1, 2019) included in the work programme of the EURL for metals and nitrogenous compounds in feed and food as of January 1, 2019*)	Instituto Superiore di Sanità, Roma, Italy (*until January 1, 2019*)
Animal proteins in feeding stuffs	Centre wallon de recherches agronomiques (CRA-W), Gembloux, Belgium
Additives for use in animal nutrition	The Joint Research Centre of the European Commission, Geel, Belgium
Genetically modified organisms (GMOs)	The Joint Research Centre of the European Commission, Ispra, Italy
Milk and milk products	*No longer required in the EU as of January 1, 2018*
Water content poultry meat	Board of Experts: JRC (IRMM), DG AGRI and the three NRLs**

* 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.

The designated EURLs for the substances and product groups as mentioned in Table 1.1 are responsible for (article 94 (2) of Regulation (EU) 2017/625) the following tasks insofar they are included in their work programmes (applicable as of April 29, 2018):

a. providing national reference laboratories with details and guidance on the methods of laboratory analysis and testing, including reference methods;
b. providing reference materials to NRLs
c. coordinating application by the NRLs and if necessary, by other OLs of the methods referred to in point (a), in particular, by organising regular inter-laboratory comparative testing or proficiency tests and by ensuring appropriate follow-up of such comparative testing or proficiency tests in accordance, where available, with internationally accepted protocols, and informing the Commission and MSs of the results and follow-up to the inter-laboratory comparative testing or proficiency tests;
d. coordinating practical arrangements necessary to apply new methods of laboratory analysis or testing, and informing NRLs of advances in this field;
e. conducting training courses for staff from NRLs and, if needed, from other OLs, as well as experts from third countries;
f. providing scientific and technical assistance to the Commission, within the scope of their mission;
g. collaborating within the scope of their mission with laboratories in third countries and with the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA);
h. where relevant for their area of competence, establishing and maintaining up-to-date lists of available reference standards and reagents;
i. where relevant for their area of competence, cooperate among themselves and with the Commission, as appropriate, to develop methods and testing of high standards.

Furthermore (article 94(3) the EURLs shall publish the list of NRLs designated by Members States in accordance with article 100 (1) of Regulation (EU) 2017/625.

1.1.3 National Reference Laboratories

RIKILT is the designated NRL for many chemical contaminants and residues (see the Introduction), GMOs, animal proteins, milk and poultry meat. A working plan describing the tasks for 2018 has been drafted in 2017. In addition, budgets for personnel, and facility and equipment costs have been drawn up. The working plans for 2018 have been positively reviewed by the Client Consultation Board

(consisting of employees of the NVWA, the Ministry of Agriculture, Nature and Food Quality (LNV) and by the Ministry of Health, Welfare and Sport (Medical Care) (VWS)) and has been approved by LNV. Working plans are based on NRL tasks as described in Regulation (EU) 2017/625 (applicable as of April 29, 2018).

As laid down in article 101(1) of Regulation (EU) 2017/625, these tasks are:

a. collaborate with EURLs, and participate in training courses and in inter-laboratory comparative tests organised by these EURLs;
b. coordinate the activities of OLs designated in accordance with article 37(1) with a view of harmonising and improving methods of laboratory analysis and test, and their use;
c. where appropriate, organise inter-laboratory comparative testing or proficiency tests between OLs, ensure an appropriate follow-up of such tests and inform the CA(s) of the results of such test and follow-up;
d. ensure the dissemination to the CA(s) and OLs of information that the EURL supplies;
e. provide within the scope of their mission scientific and technical assistance to the CA(s) for the implementation of Multi Annual National Control Plans and coordinated control plans;
f. where relevant, validate reagents and lots of reagents, establish and maintain up-to-date lists of available reference substances and reagents and of manufacturers and suppliers of such substances and reagents;
g. where necessary, conduct training courses for the staff of OLs designated under article 37(1).

Although the article describing the tasks of a NRL in Regulation (EC) No 882/2004 (article 33) was explicitly repealed as of April 29, 2018 by Regulation (EU) 2017/625, the article in Directive 96/23/EC (article 14) describing tasks of a NRL in the context of this Directive was not repealed (and will presumably apply until December 14, 2019). 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 organised 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.
The formal designation by LNV and VWS of RIKILT as NRL for the substances and products as mentioned in the Introduction was published in the 'Staatscourant' in the spring of 2018. In some cases the NRLs are mentioned in EU legislation. RIKILT has been mentioned as the 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).

1.1.4 Official Laboratories

Pursuant to Article 12 of Regulation (EC) No 882/2004 (and article 37 of Regulation (EU) 2017/625), the competent authorities are to designate OLs authorized to perform analyses of samples taken within the context of official controls. These laboratories are termed 'official laboratories' in Regulation (EC) No 882/2004 and Regulation (EU) 2017/625 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'. Of course, these accreditation requirements also apply to NRLs.

1.1.5 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). The new OCR specifies that all methods of analysis used for the official control should be accredited. For various compounds and products, specific provisions have been laid down in EU legislation concerning sampling and requirements for analytical methods. Table 1.2 shows an overview of this legislation.

Table 1.2 *List of documents with requirements for methods of analysis used in the official control*

Act/Document	For contaminant/residues/products
Regulation (EU) 2017/644	Dioxins, dioxin-like and non-dioxin-like PCBs
SANTE/11813/2017	Residues of plant protection products (all matrices)
Regulation (EC) 401/2006	Mycotoxins in food
Regulation (EC) 333/2007	Lead, cadmium, mercury, anorganic tin, 30MCPD, PAHs and melamine in food
Regulation (EC) 2074/2005	Marine biotoxins Some milk parameters
Decision 2002/657/EC	Residues of veterinary drugs and hormones (Directive 96/23/EC)
Regulation (EC) 641/2004	GMO
Regulation (EC) 619/2011	Low level presence (LLP) of GMOs in feed
Regulation (EC) 543/2008	Water content poultry meat
Regulation (EC) 273/2008	Quality parameters milk and milk products
Regulation (EC) 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

Coordinator: Stefan van Leeuwen

2.1 Activities within the EURL-NRL network

2.1.1 Participation in EURL-NRL workshops

Two EURL workshops have been organised by the EURL (CVUA, Freiburg), one in Dublin (State Laboratory, Ireland) and the other in Freiburg (Germany). At the 1st workshop (Ireland, 8/9 May 2018), Frans Verstraete (DG-Santé) updated the network on current developments at the Commission and at EFSA. The results of the proficiency tests on the cattle liver (PT 2017-2) and soy bean meal (PF 2018-1) were discussed. The Dutch NRL presented some work on the long term performance of dioxin analysis. Furthermore, the progress/state-of-play on the CWGs of BFRs and the congener patterns were discussed, as well as the establishment of the CWG on PFASs analysis. In addition, presentations were given on the PFASs analysis in Greece, results from a global proficiency test in the framework of UNEP and the proficiency test on bioanalytical screening techniques for dioxins and dl-PCBs. The workshop concluded with a (laboratory) visit to the Irish NRL (State Laboratory).
At the 2nd workshop (EURL, Freiburg, 30-31 October 2018) Frans Verstraete (DG Santé) elaborated on RASFF notifications and developments at DG-Santé. Verstraete also updated the network on the progress regarding the EFSA opinions for PFAS and dioxins. In 2018, there were 16 RASFF notifications, being on ndl-PCBs in chicken eggs (n=2), in mixed feed, sugar beet pulp, on sunflower cake and on dioxins and PCBs on protein mix for fish, calcium chloride minerals, chicken breast, sunflower fatty acids, rapeseed (n=2), cobalt carbonate, feed premix, complementary feedstuff, bentonite and sunflower cake. Ron Hoogenboom (RIKILT) discussed the EFSA opinion developments on dioxins. The revised TWI will be 7-fold lower compared to the current TWI. It is up to the commission to decide if maximum limits also need to be lowered. On the other hand, there's a debate on the TEF factor of PCB-126, which may higher than relevant from a toxicological point of view. If the TEF factor for PCB126 would be lowered, than there's maybe no need to revise MLs. At the time of the meeting, the commission did not yet take a position on either the MLs or PCB-126. Ron Hoogenboom also updated the network on the EFSA opinion on PFOS and PFOA. Due to new toxicological information that came available over the last years, the 2008 derived TDIs will be lowered substantially to TWIs, being 13 ng/kg bw week for PFOS and 7 ng/kg bw week for PFOA. It is likely that more effort on the monitoring of PFASs is needed in the next years. Possibly, MLs will be set by the Commission, although at the time of the meeting, the Commission did not yet take a position on this. Furthermore, the results of the PTs were discussed. In addition, the network was updated on the progress in the CWGs (see below).

2.1.2 Participation in working groups

The Dutch NRL participated in three core working groups (CWGs). The CWGs on dioxin patterns, on determination of BFRs and on determination of PFASs. The CWG on dioxin patterns got together in June 20 in Athens (Demokritos). At the meeting, the state of play of collection of patterns in the database was discussed. The database currently contains 280 datasets, of these 172 were assigned to primary and 102 to secondary contamination sources, as well as several software tools to evaluate unique patterns. Primary contamination sources are divided in thermal processes, chemical synthesis and biogenic sources. Secondary contamination sources are products, contaminated soils, feed and food. In these cases the time after contamination plays an important role, because the pattern can change considerably over time. The CWG discussed, if the congener pattern data base in combination with software tools for evaluation should be freely available, e.g. on the website, or will be restricted only for use within the EURL/NRL network. The CWG decided to distribute the data base and software for the moment only within the EURL/NRL network. An additional agreement could be set up which

covers the dissemination to third parties in the future and the end of the ability to use the database after leaving the network. The WG decided to address collection of additional data and individual results, the sharing of the data in the network and training of NRLs in a small committee. At that meeting it was concluded that the work of this CWG was finalised and only the work of the small committee continues.

The CWG BFRs met on June 19 in Athens. The current activities at EFSA were discussed. It was decided by EFSA to install a BFR working group that will make full updates of all current opinions except for PBBs. All opinions should be finalised by 2021. Chair of this EFSA working group is Christian Vlemminx. Several laboratories presented their methods for PBDEs in serum (NFA, Sweden), PBDEs in food (BfR, Germany) and a presentation on the combined automated clean-up of the dioxins, PCBs and PBDEs in the DexTech system. Analytical issues were discussed including avoidance of blank contributions, extraction, clean-up and GC-analysis. Unlike dioxins and PCBs, several (but not all) BFRs are less stable and may degrade under sulphuric acid treatment (clean-up) or during GC-analysis. Measures were discussed to minimise these degradation effects. An additional half-day meeting took place at the EURL (Freiburg, 31 October 2018) where analytical criteria were discussed. In addition a list of new BFR analytes was discussed, as well as the next PT on BFRs and a BFR analytical training at the EURL.

A CWG was installed on PFASs in 2018, and RIKILT will join that CWG in 2019.

2.1.3 Participation in proficiency and comparative tests

The Dutch NRL has participated in the PTs on food and feed organised by the EURL on dioxins and PCBs. In addition, the NRL participated in PTs organised by Folkehelsa (Norway) and QUASIMEME. For dioxins and PCBs, the EURL distributed a soybean meal and a beef sample. Folkehelsa distributed a fish oil, salmon and reindeer sample. On a TEQ basis (dioxin-TEQ, total-TEQ) and ndl-PCB basis, 16 out of 18 submitted results were satisfactory (between -2 and +2) and two results were unsatisfactory. Follow-up actions were taken to find the source of these two deviating results. It turned out that one of the results was deviating due to a reporting unit error: the result was reported as ng/g whereas pg/g should have been reported (1000 fold higher). On an individual congener basis, 208 results were submitted to EURL and Folkehelsa, and 175 results received a satisfactory z-score. 33 were unsatisfactory, and 30 out of these were caused by the unit error as discussed above or by the LOQ issue (Folkehelsa). Here the problem rose 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 Folkehelsa. As this issue also rose in earlier years, a complaint was filed to the provider of the PT. Following an unsatisfactory reply, the Dutch NRL decided to discontinue participation in this specific PT.
For the BFRs, the NRL participated in PT tests organised by the EURL (Soybean meal, beef), Folkehelsainstitutet (reindeer, salmon and fish oil) and Quasimeme (fish and fishery products). Data was submitted on PBDEs. Of the 73 submitted data, 9 were unsatisfactory (z>2). Several unsatisfactory scores were caused by an artefact, meaning that the LOQ value submitted by RIKILT was taken as a real measured value by the PT provider, as discussed above. Due to the higher LOD, we received a z-score >2. A complaint was filed to the provider, because this occurred several years in a row. Following an unsatisfactory reply, RIKILT decided to discontinue participation in this specific PT. Of the unsatisfactory z-scores, eight called for action (matrices beef and fish). Evaluating the data showed that an underestimation was found compared to the assigned values. The investigation into the error source has started and the results are expected early 2019.
For PFAS, the NRL participated in three studies of QUASIMEME, which focussed in fish and fishery products. Of the 24 reported numbers, 4 z-scores had a z-score >2. Proposed actions to identify the error source are (i) calibration in the matrix (matrix-matched) rather than in solvent; (ii) matching the calibration curve better to the concentration levels in the samples and (iii) choosing different ions (m/z 99) for quantification of PFOS. The results of these tests are expected early 2019. Note: Actions ii and iii resulted in better z-scores.

For chloroparaffins, the Dutch NRL has not participated in a PT, because the staff involved left RIKILT. As of the 1st of January 2019, a new scientist started on this subject and it is expected that RIKILT can participate in new PTs again in 2019.

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. Their results were good in all cases, although a slight negative bias for the PCB-TEQ and the dioxin-TEQ was observed in all cases. Two samples of 2016 and 2017 were repeated again in 2018, to monitor the performance (reproducibility) over time. The reproducibility was good and met the EU criteria (EU 2017/644) of <15% for dioxin-TEQ and total-TEQ for both samples included in this study aspect. Though in one of these cases the RSD_R was only just under 15% due to a biased result that was submitted earlier (2017). Without this result, the RSD_R would in this case lower to 4.9%, which excellently meets the criteria. This was reported back to the OL.

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

The Dutch NRL has been frequently supported of the ministries of agriculture, nature and food quality (LNV), of health, welfare and sports (VWS), NVWA and RIVM with regards to EFSA opinions published in 2018 on dioxins and PFASs, on dioxins and PCBs in fish, on PFASs in fish, and on analytical methods for GenX (a specific PFASs compound) in food and food and feed in general. Support was further given on the interpretation of analysis reports of commercial laboratories in specific cases.

2.4 Contacts with other NRLs

The Dutch NRL contacted the EURL during 2018 to discuss 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 Dioxin 2018 Conference (Fielder H, Mailisch R, et al., 2018). There has also been contact with other (non-NRL) laboratories on their experience with of analytical systems prior to the purchase of new systems by the Dutch NRL.

Fiedler, H, Malisch, R, Schachtele, A, Hoogenboom, R, van Leeuwen, S, Stephanowitz, R, Knetsch, G (2018), Pattern database for identification of sources and transfers of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls, Dioxin2018, Krakow, Poland. Dioxin 2018 conference.

3 National Reference Laboratory Pesticides in products of animal origin

Coordinator: Hans Mol

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 held 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. RIKILT is the Dutch NRL only for AO, so only one meeting was attended by the Dutch NRL as described in this report.

In 2018 a meeting was held on 9-10 October in Freiburg, Germany which was attended by RIKILT. RIKILT shares the NRL task AO with NVWA who 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 test, and to present the EURL program and activities for the next year.

The items presented and discussed are briefly summarised below:

- News and updates from the Commission and EURL (Ralf Lippold). This mainly concerned the EU coordinated multiannual control programme (MACP) regarding products of animal origin. Through this programme the EU systematically collects residue data from all member states. Each member state is obliged to generate data on a certain number of samples of the indicated animal products and analyse them on specified pesticides. This is done as part of, or in addition to, their own national monitoring/control programmes. The MACP is updated annually. The animal products included are cow's milk and swine fat for 2019, and poultry fat and bovine liver for 2020. The pesticides to be included are a number of no longer used but persistent organochlorine pesticides such as DDT, and a number of pesticides from the classes pyrethroids and organophosphorus pesticides. In addition, several 'newer' pesticides are included, such as famoxadone and indoxacarb. Several other pesticides are considered for inclusion in the mandatory scope, amongst others azoxystrobin, pendimethalin, carbendazim, chlormequat, fluazifop (-P), glufosinate, and mepiquat. For glyphosate, the residue definition will be extended with AMPA. Further details can be found in SANCO/12745/2013 rev. 10(3) 26/27 Nov 2018.

The EURL presented on an initiative to facilitate NRLs with standards of pesticides and relevant metabolites. They made an inventory on availability of commercial mix-standards containing the pesticides from the MACP programme, and asked input from the NRLs on their wishes.

The EURL for pesticides not amenable to multi-residue methods (EURL-SRM) discussed the issue that many laboratories do not cover the entire scope of the MACP. Especially in case of pesticides that need a single residue method, the number of samples analysed by member states is low or zero. The issue increased, ever less coverage of the scope, with extension of the residue definition to metabolites, which in several cases are the main residues in products of animal origin. The determination of the metabolites is often challenging because analytical reference standards are not always readily available, and dedicated analysis methods or extra steps in the sample preparation are required. The EURL-SRM is in the process of developing and validation of methods for these pesticides/metabolites.

- Proficiency test organised by the EURL. Each year the EURL organises a proficiency test to assess the performance of analyses done by the National Reference Laboratories (NRLs) and Official Laboratories (OLs). In 2018 the matrix was milk powder. The sample provided to the laboratories contained a number of pesticides from a target list. The target list included 60 pesticides that needed to be included in the scope of analysis, and in addition 50 pesticides to be included on a voluntary basis. In total ten mandatory and eight voluntary pesticides were present in the material. Levels ranged from 0.025 to 0.13 mg/kg. In total, 117 laboratories (NRLs and OLs, plus several laboratories from third countries) participated. For the mandatory pesticides, the percentage of laboratories that performed satisfactory ranged from 86% to 96%. The voluntary pesticides were covered by a lower number of laboratories. For those that did included them in the scope, satisfactory performance was obtained by 76% to 93% of the laboratories. As measure for the interlaboratory variability, the robust relative standard deviation was used which varied from 10% to 25%. The overall performance of the laboratories for this PT was above average. For the results of the Dutch NRL see 3.1.3

- Presentations by delegates from three NRLs (Cyprus, Romania, Norway) on their method used for analysis of milk (powder). The three laboratories used different methods. They were all presented and discussed in detail. The presentations showed that good results can be obtained by different methods.

- Looking back at the fipronil incident. Presentations were given by the EURL, and the NRLs from Belgium and Poland. An overview of the issue, responses and follow up by the EURL and several member states was presented. This included initiation of ad-hoc monitoring programmes for fipronil and other antiparasitics which include substances (once) registered as pesticides (acaricides/insecticides) and/or veterinary drugs. The results of this can be found in the EFSA Journal 2018;16(5):5164. Besides frequent detection of fipronil (/sulfone) the other antiparasitics were not found, except for two incidentally detects of amitraz. Also methods for analysis were presented and discussed.

- Theme day: application of high resolution/accurate mass MS for pesticide residue analysis. Five presentations were given (including one by Paul Zomer from RIKILT and one by Andre de Kok from NVWA) on technical aspects and experiences with liquid chromatography and gas chromatography combined with high resolution mass spectrometry (LC-HRMS, GC-HRMS). These techniques are maturing and have potential to be used as alternative to the established techniques (LC and GC with triple quadrupole MS, LC-MS/MS; GC-MS/MS). The advantage is that it is much easier to cover high numbers of pesticides in one measurement, and that it is possible to re-investigate existing data files for additional pesticides without the need to re-analyse the sample. Such feature would have been highly favourable in the fipronil incident. A disadvantage of the relatively new technology is that the sensitivity is approx. 5-10x worse compared to the established LC-MS/MS methods, which can be an issue for certain pesticides that have very low regulatory limits. Nevertheless, it is foreseen that in time, when existing instruments need to be replaced, HRMS instruments will gradually replace triple quadrupole instruments.

3.1.2 Participation in working groups

RIKILT is member of the advisory group on provision tests organised by the EURLs and of the analytical quality control-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'. A meeting in Madrid (27-28 June) was attended in which results of proficiency tests organised in the first half of 2018, and the ones to be organised in 2019 were discussed. In addition, a start was made with inventory of comments on the current guidance document, in anticipation of the revisions to be done in 2019.

3.1.3 Participation in proficiency and comparative tests

The Dutch NRL participated in two proficiency tests in the domain of pesticides in products of animal origin. One concerned milk powder, organised by the EURL in April. In the milk powder sample, 18

pesticides were present (ten from a mandatory scope, eight from a voluntary scope). All pesticides were found except for one pesticide from the voluntary scope (fenpyrazamine) that was not included in the method used by RIKILT. The concentrations were within the acceptable range in all cases (z-scores within -2 and +2). The other proficiency test dealt with fipronil and fipronil sulfone in three samples of eggs and three samples of chicken manure, organised by the PT-unit of RIKILT. Fipronil/sulfone was present in two egg samples (0.0056 and 0.011 mg/kg), and also in two manure samples (0.0044 and 0.012 mg/kg). In all cases fipronil/-sulfone was satisfactory quantified (z-scores within -2 and +2). Based on this, good analytical performance was demonstrated and no follow up actions needed.

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, one sample of milk powder from a previous proficiency test (Fapas) was sent to the dairy laboratory for determination of organochlorine pesticides. Results were reported to and evaluated by RIKILT, and feedback was provided to this OL.

3.2.2 Advice

RIKILT reviewed one report of a proficiency test on organochlorine pesticides and PCBs organised by Ducares for private laboratories. Input was provided to project for the NVWA: 'Prioritisation of chemical substances in animal products for national monitoring'.

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 livestock.

3.4 Contacts with other NRLs

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 international symposia: European Pesticide Residue Workshop (EPRW) in Munich, Germany 22-25th May 2018, and the 6th Int. Feed Conference, Bergen, Norway, 25-26th October 2018.

4 National Reference Laboratory Mycotoxins in food and feed

Coordinator: Hans Mol

4.1 Activities within the EURL-NRL network

4.1.1 Participation in EURL-NRL workshops

In 2017 it was announced that the Joint Research Centre (JRC, Geel, Belgium) would stop hosting the EURL for mycotoxins in food and feed, and end activities by 31/12/2017. Following a tender, RIKILT - Wageningen University & Research in the Netherlands became the new EURL, and formally started its duties per 1/3/2018. The Commission had decided to extend the domain of the EURL mycotoxins with plant toxins. Plant toxins are receiving more and more attention, as evidenced by a number of EFSA opinions and initiation of establishment of regulatory limits.

In 2018 a workshop organised by the EURL for mycotoxins and plant toxins in food and feed (RIKILT, Netherlands) was held on 9-10th October in Wageningen. 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. Members of the Dutch NRL also attended this workshop.

Below the items presented and discussed during the EURL Mycotoxins & Plant Toxins workshop are briefly summarised:

- Introduction of the new EURL (Monique de Nijs). A presentation on the organisation and tasks of the new EURL was given. The mandate and tasks according to the new regulation on official control activities were outlined (article 94.2, Regulation (EU) 2017/625). These tasks include i) disseminating analytical methods, organisation of proficiency tests, provide assistance to NRLs, provide assistance to the European Commission and other organisations where relevant, provide information on availability of analytical reference standards and certified reference materials, provide and share requirements related to legislation. The new EURL website was shown, in which information on workshops/training events, and legislation can be found, as well as a library with analysis methods and reports on previous proficiency tests. The library includes all existing documents from the previous EURL ensure they remain available to the NRL network. A follow up on the tentative EURL working programme for 2018 as presented at JRC in 2017 was given (items are described below). Also the tentative programme for 2019 was shown and discussed. Proficiency tests foreseen for 2019 were pyrrolizidine alkaloids and ergot alkaloids. Method evaluation on alternaria toxins, cyanogenic glucosides/hydrocyanic acid, and citrinin in capsules containing red yeast rice – based food supplements. Furthermore, the method performance criteria mentioned in Regulation (EC) No 401/2006 will be re-assessed in preparation to a recommendation for revision.

- News from the Commission, update on legislation, by Frans Verstraete from Directorate-General for Health & Food Safety. The current maximum level of citrinin in food supplements based on rice fermented with red yeast (*Monascus purpureus*) will be lowered from 2,000 µg/kg to 100 µg/kg. Maximum levels are foreseen for ergot alkaloids (sum of six alkaloids plus their epimers), ranging from 75-200 µg/kg (20 µg/kg for baby food). For ochratoxin A, maximum levels are under discussion for several additional foodstuffs (dried figs, cocoa, sunflower seeds). Maximum or guidance levels are foreseen for alternaria toxins. Enniatins are under consideration. Modified forms (conjugates) of zearalenone and fumonisins are not considered for regulation for the time being.

- Regarding plant toxins, adjustment in regulated levels is in progress for erucic acid. For pyrrolizidine alkaloids, regulatory measures are also in progress, based on the sum of 21 PAs (plus their N-

oxides). Possible maximum levels discussed are in the range 75-400 µg/kg for the sum (lower for infant food/tea). Regulation of tropane alkaloids will be extended to other cereals/cereal-based products. For opium alkaloids maximum levels are considered for poppy seeds (morphine equivalents = morphine + 0.2x concentration codeine) and thebaine. Maximum levels for hydrogen cyanic acid (arising from cyanogenic glycosides) in nougat, marzipan, canned stone fruits will be reconsidered. The need for maximum levels for foodstuffs like cassava and linseed is under discussion. Based on an EFSA opinion and follow up discussions, monitoring of THC (tetrahydrocannabinol) is recommended in hemp-containing food products (Commission Recommendation (EU) 2016/2115). The Commission requested EFSA opinions on glyco-alkaloids, quinolizidine alkaloids and calystegines.

- Role of LOQs in risk assessment. Ron Hoogenboom (RIKILT, EURL) explained the importance of LOQs that are fit-for-purpose for risk assessment. When health-based guidance values (acute reference dose, ARfD or tolerable daily intake, TDI) and consumption data are available, LOQs needed to allow a meaningful risk assessment can be indicated. An example was given for DON, acetyl-DONs and DON-3G: required LOQs were <50-100 µg/kg for DON, and <10-20 for the other three).

- Discussion of EURL-proficiency tests (PTs). The PTs organised in 2018 were presented and discussed. The first PT was dedicated to deoxynivalenol (DON) in wheat and maize, and also included the acetyl-DONs and DON-3-glucoside. The latter three are not yet regulated but monitoring is recommended in anticipation of possible inclusion in regulatory limits together with DON. In total, 50 laboratories participated. All participants submitted results for DON and satisfactory z-scores were obtained in almost all cases. Acetyl-DONs and DON-3G were covered by less than half, and less than one third of the laboratories, respectively. The laboratories that did have these toxins in their scope had adequate performance in most cases (≥79%). LC-MS/MS was the dominant technique used for analysis. One third of the laboratories used LC-UV after an immunoaffinity-based clean-up. The interlaboratory reproducibility (RSD_R) ranged from 14% to 28% without clear dependency regarding the mycotoxin or concentration. Results of the Dutch NRL are presented in 4.1.3.

A second PT was foreseen in 2018 on pyrrolizidine alkaloids (PAs). However, an inventory on capabilities of NRLs for the determination of PAs revealed that many laboratories did not have a method operational. Therefore the PT was postponed to 2019, and a training on this analysis was offered to the NRLs in November 2018.

- Inventory NRL capabilities. The outcome of an inventory on EURL/NRL/OL network and the analytical capabilities with respect to the determination of mycotoxins and plant toxins was presented. Not all member states appointed a NRL for plant toxins yet. The number of NRLs in each member state to cover mycotoxins and plant toxins in food and feed ranged from one to more than four. The current network comprised 47 NRLs which in turn were responsible for the performance of 175 OLs. The number of OLs per member state ranged from none to 25. The Netherlands has one NRL and two OLs. In terms of scope of analysis, the regulated mycotoxins were generally covered by all NRLs, with the exception of citrinin. Not (yet) regulated mycotoxins were only included by part of the NRLs, in decreasing order: ergot alkaloids, acetyl-DONs/DON-3G, alternaria toxins, enniatins, sterigmatocystin and phomopsins. For plant toxins, methods were operational in only part of the NRLs, in decreasing order: tropane alkaloids, pyrrolizidine alkaloids, erucic acid, hydrogen cyanic acid, harmful botanicals in feed, cannabinoids, opium alkaloids, glyco-alkaloids and quinolizidine alkaloids.

- Pyrrolizidine alkaloids. Patrick Mulder (EURL) gave a presentation on the background of these plant toxins, and analytical methods. Several options were presented: determination of individual PAs as free bases and their N-oxides, determination of total free bases after a reduction step, and a method based on alkaline hydrolysis and then determination of the four dominant necine moieties. At the moment, the first option is considered most appropriate.

- Ergot alkaloids. Aleksandrs Versilovskis (EURL) presented the applicability of an LC-MS/MS based method for the determination of ergot alkaloids in processed products (bread). The method proved to be suitable down to low µg/kg levels. Three commercially available ELISA assays for ergot alkaloids were tested as screening method. They were found of limited use at the moment.

- Cyanogenic glycosides (CNGs)/hydrogen cyanic acid (HCN). Different options for determination of HCN were presented: methods involving conversion of CNGs into HCN followed by determination of total HCN by LC-fluorescence (EN 16160:2012) and a method based on LC-MS/MS determining the intact CNGs. The latter was much faster, and gave equivalent results for non-processed products (apricot kernels, almonds, linseeds) but differences were observed for processed products (e.g. cookies).

- Relevant international projects in the field of mycotoxins and plant toxins. Several EU funded projects were presented for information, MyToolBox, MycoKey, HBM4EU. Details on these projects can be found on the websites under these names. Furthermore, an update was given on the progress in the development of standardized methods for mycotoxins and plant toxins in food and feed within the frame of CEN (European Committee for Standardization).

4.1.2 Participation in working groups

As part of the EURL task, RIKILT has initiated a working group on analytical quality control (AQC). The first meetings are foreseen in 2019. The aim of the working group is to discuss analytical issues and to provide guidance on AQC in the determination of mycotoxins and plant toxins and data interpretation.

4.1.3 Participation in proficiency and comparative tests

In 2018, the Dutch NRL participated in three proficiency test on mycotoxins or plant toxins: aflatoxin M1 in milk powder (Fapas), pyrrolizidine alkaloids and tropane alkaloids in rye flour and wheat (BfR, Germany), and cyanogenic glycosides (RIKILT PT-unit). In all cases satisfactory quantitative performance was obtained (Z-scores all within -2 and +2) in the sample materials.

4.2 Assistance to official laboratories

4.2.1 Quality control

In the Netherlands, besides by the NRL itself, official samples are analysed by two OLs. One OL specifically analyses dairy products for presence of aflatoxin M1. Both the Dutch NRL and the non-dairy-OL analyse most samples using multi-methods also covering many other mycotoxins than the regulated ones. Monitoring the performance of the non-dairy-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-OL, quality control was done by sending standard solutions and milk samples containing aflatoxin M1. Results were reported to and evaluated by the Dutch NRL, and feedback was provided.

4.2.2 Advice

Technical advice was given on the determination of tropane alkaloids and possibilities to include them in multi-methods for mycotoxins. Furthermore, there were ad-hoc contacts with the non-dairy-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 RIKILT, also the Ministry of VWS 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. In 2018 there was specific exchange of information and interpretation of data regarding hydrogen cyanide in apricot kernels, bitter/sweet almonds, and products containing these ingredients. Input was provided for a risk assessment on hydrogen cyanide in foodstuffs, done by RIVM-RIKILT ('Front office food safety') at request of NVWA-BuRO.

4.4 Contacts with other NRLs

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: 10th conference of the World Mycotoxin Forum, March 12-14, Amsterdam and 10th Int. symposium on poisonous plants (ISOPP), September 16-20, St. George, Utah, USA.

5 National Reference Laboratory Metals and Nitrogenous Compounds

Coordinator: Martijn van der Lee

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 Chemical Elements in Food of Animal Origin (EURL-CEFAO, ISS, Rome, Italy) and the EURL for Metals and Nitrogenous compounds in feed and food (EURL-MN, DTU, Copenhagen, Denmark).

The annual workshop of the EURL-CEFAO was held in Rome on October 5, 2018. The meeting was attended by forty-eight participants, representing NRLs from each EU Member State (with the exception of Malta), invited speakers and participants and staff from the EURL-CEFAO. The participants were welcomed to the last EURL-CEFAO workshop by the EURL Director, since the mandate of the EURL-CEFAO expired on December 31st, 2018. A representative of the Italian Accreditation Body (ACCREDIA) presented the revision of the ISO/IEC 17025 that was published in November 2017. The results of the two EURL-proficiency tests (PTs) that were organised in 2018 were discussed. The first PT (EURL-CEFAO 28th PT) concerned the determination of cadmium, lead, arsenic, mercury and nickel in frozen mussels. Several unsatisfactory results for nickel were observed which was attributed to the low concentration in the PT sample and the low experience of some of the NRLs with the analysis of nickel. The second PT (EURL-CEFAO 29th PT) discussed included the analytes calcium, cadmium, lead, mercury and tin in grilled turkey breast, a processed food. Besides the analytical results, the capability of the NRLs to confirm the compliance with maximum levels as mentioned in Regulation (EC) No 1881/2006 for processed food was evaluated. To confirm compliance, concentrations have to be corrected for a given moisture loss of 30%, according to article 2 of this Regulation. PT results indicated that several labs (not including the Dutch NRL) had problems with correcting for concentration changes due to processing of foods. Therefore the NRLs were instructed on how to assess the compliance with maximum levels of metals for processed food. After a presentation by a representative of the Irish NRL, the workshop was concluded with a training session on the traceability of analytical measurements given by a staff member of the ISS.

The first workshop of the new EURL for Metals and Nitrogenous in feed and food (MN) was hosted by the National Food Institute at the Technical University of Denmark (DTU) in Copenhagen on November 14 and 15. The workshop was organised jointly with the EURL-PC (Processing Contaminants) and EURL-CF (Pesticides in cereals and feed). Since 2018 was the first year of the EURL-MN, the new EURL was introduced by the head of the EURL-MN, Jens Sloth. The EURL-MN has organised two PTs in 2018, one for the determination of nickel, cadmium, lead, mercury, total arsenic and inorganic arsenic in mixed corn poultry feed (EURL-MN PT-2018-01) and one for the same analytes in chili powder (EURL-MN PT 2018-02). Results of these PTs were presented and discussed. There were several interactive sessions in which the delegates of the NRLs discussed the expectations of the EURL network, options for future PTs and future activities of the EURL-MN. Frans Verstraete (DG SANTE) presented recent and future developments of the EU Regulations for heavy metals in feed and food. The importance of monitoring nickel and arsenic concentrations in various matrices was stressed. There is limited regulation for these elements but there are concerns about potential health effects. Therefore the NRLs were encouraged to monitor nickel and arsenic concentrations in accordance with the EU Recommendations (EU) 2015/1381, (EU) 2016/1111 and (EU) 2016/1110 and to provide their data to EFSA.

The analysis of nitrogenous compounds in feed and food is within the scope of the new EURL, but only few NRLs have experience with this type of analysis. Therefore, EURL-MN staff member Kit Granby

presented an overview of the issues related to nitrogenous compounds in feed and food and the analysis of these compounds. Compounds of interest are nitrite, nitrate, melamine, urea and cyanuric acid. The Dutch NRL has analytical methods available for the latter three, the Dutch OL has experience with the analysis of the first two compounds.
The second day of the workshop was held at the facilities of DTU Food in Kgs. Lyngby, Denmark. After a plenary key-note lecture by Prof Jørn Smedsgaard (DTU Food), there were several contributions by NRL representatives. Finally, a tour in the laboratory facilities of the EURL-MN was organised for the participants.

5.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to metals in food and feed in 2018 to participate in.

5.1.3 Participation in proficiency and comparative tests

The NRL has participated in all four EURL proficiency tests mentioned previously. In addition, the NRL participated in several other internationally organised PTs (Table 5.1).

Table 5.1 *Overview of proficiency and comparative tests, NRL heavy metals.*

PT	Analytes	Matrix	z-scores
FAPAS 07305	As, Hg, MeHg	Canned fish	Between -0.2 and 0.9
FAPAS 07307	As, Cd, Pb, Hg, iAs	Rice cakes	Between -0.2 and 0.5
EUPT-SRM13	Br	Soy bean flour	-0.6
FAC-18-01	Se	Compound feed	1.8
FAPAS 07312	As, Pb, Cu	Edible oil	Between -0.3 and 0.7
EURL-CEFAO 28th PT	As, Cd, Hg, Pb, Ni	Frozen mussels	Between -0.3 and 1.3; Ni 3.7
EURL-CEFAO 29th PT	Cd, Pb, Hg, Ca, Sn	Grilled turkey breast	Between -1.1 and 0.1
FAPAS 07315	As, Cd, Pb, Hg	Offal (liver)	Between -0.3 and 0.6
EURL-MN PT-2018-01	As, Cd, Pb, Hg, Ni, iAs	Mixed corn poultry feed	Between -1.1 and 0.06
EURL-MN PT-2018-02	As, Cd, Pb, Hg, Ni, iAs	Chili powder	Between -0.28 and 0.14
FAPAS 07323	Cd, Pb, Ni, Sn	Vegetable Purée	Between -1.1 and 0.2

All results of the reported concentrations in the PTs mentioned above were satisfactory (z-scores between -2 and +2), with the exception of nickel in frozen mussels (EURL-CEFAO 28th PT). The ICP-MS method for nickel was evaluated and the two jars of PT material that were received were reanalysed. From the experiments performed it could be concluded that the PT material in one of the jars was contaminated with nickel and this led to the z-score of 3.7. No issues related to the performance of the method were identified during the evaluation of the ICP-MS method.

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 taken over the measurements since, and thus the responsibility for the analysis of 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 organised by the EURL, FAPAS and other PTs, and the z-scores were evaluated by the NRL and OL. Furthermore, for quality assurance, the Dutch NRL has prepared a comparison study based on the analysis of heavy metals in milk samples. The samples were handed to the OL and results were discussed during a NRL OL meeting.

OL milk and milk products
Analyses for the competent authority (CA) for milk and milk products are carried oud 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 that were sent to the OL. The results of the analysis by the OL were discussed with the CA milk and reported.

5.2.2 Advice

Since the merger of the laboratories of the OL and the NRL, advice on analytical measurements, quality and measurement strategies are given 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 organised two times a year by the NRL. In 2018, the results were good and no specific advice was given.

5.3 Scientific and technical support to the competent authority

As mentioned in 5.1.1 monitoring of elements that are not yet regulated but are of concern with respect to potential health risks are an important topic in the EURL-NRL network. Therefore, nickel has been added to several ICP-MS methods for food matrices by RIKILT. In the context of Recommendation (EU) 2016/1111 the Dutch NRL together with the OL analysed a total of 177 food samples for the nickel content. The results of this survey were reported to the CA.

5.4 Contacts with other NRLs

During the EURL workshops, relationships with other NRLs were maintained. Furthermore, the Dutch NRL participated in a pre-test of a collaborative trial organised by the German NRL for a method on mercury in food and discussed the method and results of the collaborative trial. Work related to selenium speciation and arsenic speciation was discussed with the Belgian NRL.

6 National Reference Laboratory for Processing Contaminants (EURL-PC)

Coordinator: Martijn van der Lee

6.1 Activities within the EURL-NRL network

6.1.1 Participation in EURL-NRL workshops

In 2018, the Dutch NRL participated in the first Workshop of the EURL for Processing Contaminants (PC) hosted at the National Food Institute at the Technical University of Denmark (DTU) on November 14 and 15. The workshop was organised jointly with the EURL-MN (Metals and Nitrogenous Compounds in Feed and Food) and EURL-CF (Pesticides in cereals and feed), both hosted by the National Food Institute (DTU Food). Twenty-six participants attended the event, representing 24 National Reference Laboratories (NRLs), the Directorate General Health and Food Safety (DG SANTE) and staff from the EURL-PC. All delegates of the NRLs and the Directorate General were welcomed at the meeting by the EURL Director. During the meeting representatives from the EURL-PC gave presentations on the EURL-PC with respect to their tasks followed by presentations from the EU member states of their NRLs. The outcome of the proficiency test on acrylamide in bread organised by the EURL-PC in 2018 was presented and discussed. Two NRLs representatives, including the Dutch NRL, gave presentations on their current activities within polycyclic aromatic hydrocarbons in food supplements and herbs. A representative from DG SANTE presented the recent and expected future developments of the EU legislation in the area of competence of the EURL-PC. Discussion sessions were organised where the participants discussed their expectations to the new EURL-PC, needs for the NRLs as well as provided suggestions for future PTs and other activities in the network.

The second day of the workshop was held at the facilities of DTU Food in Kgs. Lyngby, Denmark. A welcome address was given by the Provost of DTU followed by a key-note lecture by Prof Jørn Smedsgaard (DTU Food). Finally, a tour in the laboratory facilities of the EURL-PC was organised for the participants.

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 the Dutch trade/branch organization for dried herbs and food supplement processing factories, the OL a commercial contract laboratory and the NRL. In 2017 a PT was organised by the NRL to find out if the results of analysis of participating laboratories are comparable. More than 20 laboratories participated in this PT and in 2017 a report was written on this PT. In 2018 the report was discussed in the working group. The Dutch NRL representative also presented the outcome of this PT during the EURL-NRL meeting in Denmark. The most important outcome of the PT was that harmonising of the extraction and clean-up method could result in a more equivalent outcome of the results of analyses. The relative high variation in PAH concentrations reported by the different laboratories in the same samples of herbs could be caused by the different extraction methods used.

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 (MOSH-MOAH), 2- and 3-mcpd esters and glycidyl esters, acrylamide and furans are included. The Dutch NRL participated also in PTs on these contaminants. In 2018 the NRL participated in the PTs organised by EURL, FAPAS and PROOF-ACS, test and results are presented in the following table.

Name of PT	Topic	z-score
EURL	Acrylamide in bread	-0.1
FAPAS 0673	PAH in Spirulina powder	Between 0 and 1.2
FAPAS 0676	PAH in Cocoa butter	Between –0.6 and 0
FAPAS 0677	PAH in Smoked fish	Between 0.3 and 1.8
FAPAS 2653	MCPD in Potato chips	Between -0.5 and 0.6
PROOF-ACS	MOSH-MOAH in Chocolate muesli	Between 1.2 and 7.1*
PROOF-ACS	MOSH-MOAH in Rapeseed oil (spiked)	No scores; Assigned values below LOQ

* Actions were taken by NRL. Samples were reanalysed and method is evaluated.

6.2 Assistance to official laboratories

6.2.1 Quality control

On request of the Dutch CA for milk and milk products, the NRL analyses 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 2018, 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, the extraction of PAH and the determination of PAH in herbs and food supplements were discussed with the representative of the OL. The NRL analysed official control samples of milk for the CA, 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 OL, NRL and commercial contract laboratories in the Netherlands, the CA NVWA has started a working group on this topic. In 2017 and 2018 this workgroup held several meetings and as a result of those meetings RIKILT organised a special PT on PAH in herbs and food supplements (see above). In 2018 the report of this ring trial is published. Participating laboratories were asked to fill in a questionnaire to investigate if there were any relations between the method used and their reported results. The CA, the NRLs and EURL were informed on the outcome of this PT as well as the outcome of the study based on the answers given in the questionnaire on the method used.

6.4 Contacts with other NRLs

During the year 2018 the Dutch NRL did have contact with EURL about the analysis of PAH in herbs and food supplements. During the EURL workshop, the relationship with other NRLs was maintained. Some NRLs, who did not participate in the PT on herbs and food supplements before were contacted and asked to analyse the same material on PAHs, and send in their results together with information on the analytical method used to RIKILT. The new analytical results of these laboratories will be evaluated in 2019.

7 National Reference Laboratory Marine Biotoxins

Coordinator: Arjen Gerssen

7.1 Activities within the EURL-NRL network

7.1.1 Participation in EURL-NRL workshops

The EURL annual meeting was held in October 2018 in Vigo, Spain. In this meeting results of various proficiency test (PT) studies and other issues were discussed as described below. Paolo Caricato of DG-SANTE attended this meeting to share the vision of the EU Commission on several topics.

Due to decision of the UK to leave the EU, the tasks of the EURL for the monitoring of bacteriological and viral contamination of bivalve molluscs, previously assigned to CEFAS in the UK, have been reassigned to the EURL for Marine Biotoxins. 2019 will be a transition year and progress on these additional tasks of the EURL Marine Biotoxins will be included in the next annual workshop meeting. Laboratories involved in the classification and monitoring of bacteriological and viral contamination will be invited for this meeting.
The EU Commission and the Irish NRL gave a presentation on the analysis of amnesic shellfish poisoning (ASP) toxins in scallops. The edible part of scallops (adductor muscle and gonad tissue) rarely exceed the legal limit, while non-edible parts can be highly contaminated. According to the Commission Decision 2002/226/EC the ASP concentration in the whole scallops should not exceed 250 mg/kg, whereas Regulation (EC) No 853/2004 states that the maximum concentration in the edible parts should not exceed 20 mg/kg. This is inconsistent with how the legislation for other toxin classes, paralytic shellfish poisoning (PSP) toxins and lipophilic shellfish toxins is interpreted in practice; maximum concentrations are applicable to whole shellfish bodies. Related to this subject, it was discussed how the opening and closing of off-shore scallop harvesting areas could be applied, as these are non-official production areas which are difficult to open and close. Furthermore sampling in these areas is time consuming and costly.

The EURL informed the NRLs that the Lawrence method (using HPLC-FLD) is now the reference method in the EU for PSP testing instead of the mouse bioassay (MBA). This implies that the EURL will discontinue their MBA activities, and therefore the accreditation for this method will not be renewed and no PTs will be organised for the NRL network for the MBA. Some member states regard the Lawrence method as laborious and a time consuming, however the EURL indicated that the Lawrence method can also be used as a rapid screening tool. The NRLs are invited to attend a training in the application of the method for rapid screening.

The Regulation (EC) No 854/2004 will be replaced at the end of 2019 by Regulation (EU) e2017/625. The EURL and NRLs discussed an implementing act in which the use of animal tests for the monitoring of emerging toxins is mentioned with DG-SANTE. The EURL and NRLs argued that the phrasing proposed by DG-SANTE would allow the use of the MBA, as well as chemical methods or alternative methods such as bioassays, for periodic monitoring of emerging toxins. The EURL and NRLs think that there is still too much emphasis on the MBA, and that only chemical and alternative methods should be mentioned in the act, as the MBA is limited in its ability to identify all toxins in a given sample. In reply, DG-SANTE stressed that it is difficult to make legislation when agreement between 28 member states is required.

Another important issue was the monitoring of tetrodotoxins (TTX) in shellfish by various member states. In The Netherlands, the maximum level found was 54 µg TTX/kg, 132 samples were analysed. In Spain, 1351 samples from the Galicia region were analysed, three samples contained very low TTX concentrations (< 3 µg/kg). Ireland performed analyses on 503 archived samples from 2012-2016, in

all samples concentrations were below the LOQ of 40 µg TTX/kg. Norway and Italy tested a small number of samples, Norway did not detect any TTX and Italy found concentrations up to 216 µg TTX/kg is samples from along the Adriatic coast. France performed a survey in 70 gastropod samples, in none of the samples TTX was detected.

Finally, the EURL stated that the guideline document for harmonisation of phytoplankton monitoring is close to finalization, last rounds of review are planned for early 2019.

7.1.2 Participation in working groups

The NRL participated in two EURL advisory board meetings. In March, a first advisory board meeting was held in Porto, Portugal. During this meeting the various guidelines were discussed; the marine biotoxin guideline, the phytoplankton guideline and the guideline for classification of production areas. All are now the responsibility of the EURL. A discussion was held on the most effective consultation of member states (NRL and competent authorities) to draft the marine biotoxin guideline. A second advisory board meeting was held prior the EURL-NRL workshop in Vigo. During this meeting the outline and timeline of the marine biotoxin guide was drafted and this outline was presented to the NRLs a few days later.
The working group on phytoplankton monitoring did not reconvene in 2018. However various email discussions were held, in total 11 review rounds have been finalised. An expert from Wageningen Marine Research represents the Dutch NRL. The document is planned to be finalised in the course of 2019. Various chapters have been drafted on sampling plans, sampling and transport, counting methods, interpretation of data and quality control. For the sampling plans, the EU Regulation is vague. Therefore, this guideline will be the key to harmonisation of the sampling plans. Already existing guidelines are adopted in the new guideline. For example, for sampling and transport, mostly EN15972:2011 and EN15204:2006 will be followed.

7.1.3 Participation in proficiency and comparative tests

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 ASP group as most laboratories had satisfactory results. The NRL had z-scores below |2|, which indicates that the method is performing satisfactorily. Only the results of the EU reference method, HPLC-UV, can be submitted to the EURL. The samples analysed by HPLC-UV were also analysed by the NRL with a LC-MS/MS method which is used routinely for ASP monitoring. Based on our own calculations also this test is performing satisfactorily.

Next the results of the PT on PSP toxins in shellfish were discussed. In general the number of non-satisfactory results are higher for PSPs than for the other toxin classes. For the total toxicity sample 1, 3 had a good z-scores of 1.2 for both samples, for sample 2 an unsatisfactory z-score of 2.8 was obtained. For the individual toxins unsatisfactory results were obtained for GTX2&3 (z-score sample 1 3.2; sample 2 3.5), GTX1&4 (z-score sample 2 2.2) and dcGTX2&3 (z-score sample 3 3.3). All errors can be related to the recovery correction which is applied. When re-calculating the results without recovery correction, results for GTX2&3, GTX1&4 and dcGTX2&3 are sufficient and with z-scores < |2|. However, after re-calculation using the recovery correction dcNEO and GTX6 gave unsatisfactory results (z-scores -2.9 and -2.6 in sample 3). Both errors can be related to the poor recovery of the ion exchange-SPE cartridges used. Although the method used is a CEN standardized procedure, it seems that poor recovery is obtained due to variations between batches of SPE cartridges. Further research in early 2019 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 our routine control program as screening method. All positive samples analysed with this method were identified as suspect and therefore the performance of the screening method is satisfactory.

Most laboratories performed satisfactorily in the PT on lipophilic marine biotoxins. In total three samples were analysed by LC-MS/MS. The NRL performed satisfactory for the total toxicity content in all samples (z-scores < |2|). For sample 1 a z-score of -0.5 was obtained for the OA group toxins. For

Sample 2 a z-score of -0.4 was obtained for the OA group toxins and -0.3 for the yessotoxin group toxins. For Sample 3 a z-score of -0.5 was obtained for the OA group toxins, 0.3 for the azaspiracid group toxins and 0.3 for the yessotoxin group toxins. Also for the individual analogues the NRL obtained good results.

A PT was organised for TTX, however the number of participants was too small for statistical evaluation. The results of the PT show that there is quite some variation between the laboratories. The EURL will organise a LC-MS/MS expert WG meeting to discuss how the method variation can be reduced. Furthermore, a PT for TTX is foreseen for 2019 as well.

7.2 Assistance to official laboratories

7.2.1 Quality control

Besides the NRL, there is one OL in The Netherlands. 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. Results of these PTs were submitted to the NRL at the end of 2018, and will be evaluated early 2019.

7.2.2 Advice

The results of the 2018 Quasimeme PT assessment of the OL will be evaluated by the NRL together with the OL early 2019. Based on this evaluation, assistance will be given if needed. The OL was informed of the activities of the NRL in 2018 during the annual NRL meeting held early 2019.

7.3 Scientific and technical support to the competent authority

In 2018, there were several contact moments between the NRL and the Dutch competent authority, the NVWA. The NRL formally advised the NVWA to support the deregulation of pectenotoxins. Furthermore, the NRL was asked by the NVWA to give their opinion about labelling certain fish species with 'might contain ciguatera'. According the NRL this approach will not be feasible and the NVWA supports this opinion. Finally, a report on the relationship between toxic algae and toxins in shellfish was finalised and offered to the NVWA. This report was made publically available in the beginning of 2019.

7.4 Contacts with other NRLs

As the number of issues with marine biotoxins in shellfish was limited over the course of 2018, only limited interaction occurred between the various NRLs. The Dutch NRL was contacted by various NRLs to supply them with TTX contaminated shellfish, but due to the lack of contaminated material, no large portions of shellfish samples could be distributed to the other NRLs.

8 National Reference Laboratory NRL (96/23/EC)

Coordinator: Johan Lasaroms

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 12th until the 14th of June organised by 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 organised proficiency testing, developments in the field of residues for which BVL is responsible (nitroimidazoles, coccidiostats, NSAID's, beta-agonists and anthelmintics)), also the following topics were presented: Multi-screening methods with HRMS instruments; Synthesis of standards and quality control; laboratory-comprehensive validation study of the NSAID project and future plans and discussion on the Revision of Decision 2002/657/EC (in particular the points 'decision limit', 'detection capability' and 'method validation'. Also the confirmation criteria were discussed).

Secondly, the NRL participated in the EURL-Workshop in Fougères, held on the 28th and 29th of June. This workshop For the Control of Antimicrobial Residues in Food from Animal was organised by ANSES. This workshop consisted of only a theoretical part. Some topics of this Workshop were: 'Overview of the PT program 2017-2018'; Control of Antimicrobial Residues by LC-HRMS – Screening and confirmation'; 'LC-MS/MS for the control of Authorized Antimicrobial residues'; 'LC-MS/MS for the Control of Banned Antimicrobial Residues'; 'Bio-Screening methods for the Control of Antimicrobial Residues' and 'Reference Testing Materials'.

Thirdly, the NRL participated the EURL-Workshop in Wageningen, held from the 15th until the 17th of October. This workshop also consisted of a theoretical part and a practical part. Some topics of this workshop were: 'Update EU-RL activities including Evaluations MS NRCP results'; 'Discussion on draft document revision 2002/657';, 'NRL open forum with topics like: - 'Myostatine a new growth promotor approach'; - 'Ion Mobility in residue analysis'; - 'rBST an update' ; -'Antedating for steroid esters'; - 'Nortestosterone and metabolites, an approach to discriminate endogenous from exogenous'.

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:

- 8th International Symposium on Hormone and Veterinary Drug Residue Analysis in Ghent, held from 22th until the 25th of May.
- The NRL participated in the meeting Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) which was held on the 5th of April.

8.1.2 Participation in proficiency and comparative tests

The NRL has participated in several proficiency tests organised by the EURLs and other international proficiency testing organizations and has obtained the following scores:

Description	Organizing institute	Z-score	Assessment
Coccidiostats in milk powder, quantitative confirmation	Ducares	Between -0.5 and 1.4	Sufficient
Coccidiostats in laying maize, quantitative confirmation	Ducares	Between -0.7 and 1.0	Sufficient
Antibiotics, coccidiostats in compound feed, (quantitative) confirmation	RIKILT	Between -1.09 and 1.71	Sufficient
Nitrofurans and chloramphenicol in porcine meat, quantitative confirmation	EURL-Anses	Between -0.54 and 1.33	Sufficient
Coccidiostats in meat (goat/sheep), quantitative confirmation	EURL-BVL	Between 0.22 and 0.95	Sufficient
Corticosteroids in bovine liver, quantitative confirmation	FAPAS	Z-score: -0.5	Sufficient
Beta-agonists in bovine liver, quantitative confirmation	FAPAS	Between -0.9 and -0.5	Sufficient
Dyes in fish, quantitative confirmation	FAPAS	Between -0.1 and 0.5	Sufficient
Stilbenes and steroids in bovine urine, quantitative confirmation	FAPAS	Between -1.9 and 0.9	Sufficient
Coccidiostats in chicken egg, quantitative confirmation	FAPAS	Between 0.2 and 0.7	Sufficient
Avermectines and anthelminthics in bovine liver, quantitative confirmation	FAPAS	Between -0.6 and 1.6	Sufficient
Dyes (specific malachite green) in fish, quantitative confirmation	FAPAS	Between -1.8 and -1.1	Sufficient
Resorcylic Acid Lactones (RALs) in bovine urine, quantitative confirmation	Progetto	Between -1.15 and -1.24	Sufficient
Beta-agonists in porcine urine, quantitative confirmation	Progetto	Between -0.76 and -0.5	Sufficient
Veterinary drugs in milk powder and feed for porcine, (quantitative) confirmation, residue level	Ducares	Between –0.9 and 1.4	Sufficient
Antibiotics in bovine meat, quantitative confirmation	RIKILT	Between -0.69 and 1.23	Sufficient
Chloramphenicol in porcine urine and meat	EURL Anses	Between -0.86 and 0.83	Sufficient
Gestagens in bovine kidney fat, quantitative confirmation	RIKILT	Between -0.41 and -0.05	Sufficient
A3 steroids in bovine urine, quantitative confirmation	RIKILT	Between -1.97 and -0.14	Sufficient

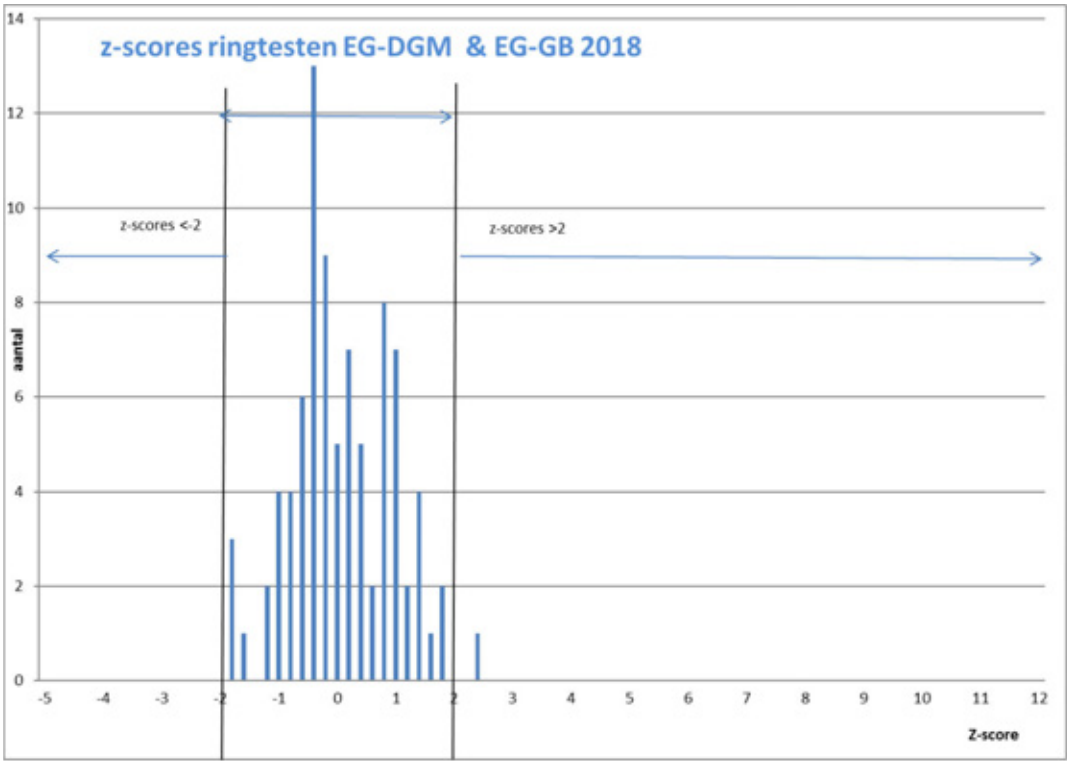

Figure 8.1 *Overview of the obtained z-scores.*

As can concluded from the table and also from the figure all the obtained results (z-scores) are sufficient, no corrective actions were needed.

In the figure there is one deviant result is present, this is originating from a proficiency test which was organised in 2016. The Dutch NRL received the report on this PT in 2018 (this proficiency test is not mentioned in the table).

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 analyses of the OLs) is only focused on the analyte/matrix combinations from Group A (prohibited substances) and group B (regulated substances), mentioned in Directive 96/23/EC. The current control program (third-line control program) includes 38 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 2018 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

In 2018 the NRL participated in three meetings for the National Plan Residue control workgroup, these meetings were held on the 26th of March, the 3rd of September and on the 3rd of December.

A National Expert from the NRL has participated in an FVO audit in China, which was held from the 2nd until the 13th of July, in order to evaluate the control of residues and contaminants in live animals and animal products including controls on veterinary medicinal products

In 2018 the NRL participated in a meeting between RIKILT and an OL with the topic 'the implementation of antibiotic analysis in dairy product which are exported to Russia', which was held on the 19th of June.

In 2018, contra-expertise was performed five times for the OL. These were for confirmation of alpha-nortestosteron in horse urine; confirmation of boldenone in bovine hair and urine; confirmation of AOZ in shrimp; confirmation of hexestrol in urine and confirmation of trenbolone in urine.

9 National Reference Laboratory animal proteins

Coordinator: Leo van Raamsdonk

9.1 Activities within the EURL-NRL network

9.1.1 Participation in EURL-NRL workshops

The annual meeting of the EURL Animal Proteins (AP) and the NRLs was organised in April in Puławy (Poland). In one and a half day a range of topics was addressed, including a presentation and discussion of the 2017 proficiency test and the poultry (chicken/turkey) PCR validation study, the effect of grinding, muscle specific peptides, and the detection of insects. The Dutch NRL was present with three experts on light microscopy and PCR. They participated actively in the discussions and in the informal lobby contacts.

The issue of hydrolysed proteins was addressed during the open discussion. Since these types of animal proteins are not included in the definition of processed animal proteins (PAPs), and in the view that the nature of the materials differs considerably from the general type of 'meat and bone meal', specific method for characterization are needed. Several NRLs indicated interest in the further development of dedicated methods. The EURL expressed to have no intention for starting activities to develop such methods. The NRLs from the member states France, Poland and the Netherlands were planning to join forces. The first expression of the cooperation was a joint publication during the Feed conference in Bergen (Norway) in October 2018.

The interlaboratory study for the microscopic detection of insects was announced during the meeting in April. The Dutch NRL participated in the study, which was held during the summer season. There were serious complications for the implementation of the method using the RIKILT glassware. This equipment is suitable for the standard detection method as included in Regulation (EC) No 152/2009 Annex VI and was tested by the EURL as best performing. However, for the specifications as set for the insect detection method standard chemical separation funnels need to be operated. The practical implementation and additional training consumed a considerable part of the financial means of this NRL project. The results were submitted to the EURL accompanied with a report of the issues encountered during the implementation of the method and examination of the samples. The report was not issued during the year 2018. One of conclusions is that a combined method for simultaneous detection of PAPs (the method currently included in the Annex) and of insects is not likely, due to both the difference between equipment and solvents, and the time stamp.

A representative of the Commission was present at the meeting and explained new developments in legislation.

9.1.2 Participation in Working groups

In the absence of official working groups RIKILT seeks cooperation on a bilateral basis.

9.1.3 Participation in proficiency and comparative tests

The final report of the annual proficiency test 2017 was released in March 2018. The PT of 2017 comprised a total of six samples. Five of these samples were considered the set for the PT, the sixth sample consisted of a fish feed with 0.5% insect meal. Of the five samples two were included twice. A set of eight jars was distributed, indicating the type of feed. Each participant was requested to perform the required analyses for each of the jars based on the type of feed. The composition of the samples, the required analyses and the overall results are indicated in Table 9.1.

Table 9.1 *Design and results of the 2017 proficiency test for animal proteins. Six different samples were included, two of them twice. All jars were labelled according to the type of feed without mentioning the contaminant. Results are indicated as sensitivity in presence or specificity in absence of the contaminant. All fish feeds contained fish material. An optimal score over all participants equals one.*

Sample no	Composition	contaminant	Method	Result			
				terrestrial animal	fish	Ruminant PCR	insect
1 (2x)	Poultry feed		Micr	1.0	0.96		
2	Fish feed	+ 0.1% porcine PAP	Micr + PCR	0.92	1.0	0.96	
3	Horse feed		Micr	1.0	1.0		
4 (2x)	Fish feed	+ pork haemoglobin	PCR			0.84	
5	Juvenile trout feed	+ 0.05% ruminant PAP	Micr + PCR	0.96	0.96	0.96	
6	Juvenile trout feed	+ 0.5% insect meal	Micr	0.92	1.0		0.16

In several cases fragments were incorrectly identified as fish material (sample 1) or terrestrial animal material (sample 6), or animal material was not detected (fish: sample 5; terrestrial animal material: samples 2 and 5). Overall the results were good. The Dutch NRL provided fully correct results for microscopy and belonged to a group of 20 NRLs which all reported a correct set of results. All errors for microscopic detection were made by five NRLs.

The presence of pork haemoglobin powder in a fish feed, which was not to be examined by microscopy according to the mandatory flow scheme, was reported as originating from ruminant by eight participants. The overall results for detection of ruminant material by PCR were correct for 21 participants out of 25. The Dutch NRL submitted fully correct results.

The presence of insect material was reported by only four NRLs (not including the Dutch NRL). This can be assumed to be caused by the situation that insect fragments do not match the usual appearance of PAPs, and detection was hampered by the method applied (Annex VI), which was not designed to select insect particles. More training and an adaptation of the method is needed.

The Dutch NRL participated in the Chicken and Turkey PCR implementation study that was sent in March 2018. Eight samples and a positive control sample were provided. Also, the cut-of had to be established. The results were reported within the timeframe. The results were presented at the annual meeting in Poland. It was concluded that the results of the validation and implementation studies of the method were satisfactory.

The samples for the annual proficiency test 2018 have been sent around in November. This test combined (again) microscopy and PCR analyses. The proficiency test of 2018 comprised a total of seven samples. Results were submitted on time. Preliminary feedback revealed no errors for both microscopy and PCR.

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 mandated competent authority (NVWA: Netherlands Food and Consumer Product Safety Authority) and to the competent authority, 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 first process for revision of the microscopic method as included in Annex VI of Regulation (EC) No 152/2009, and the monitoring of poultry material for supporting the anti-cannibalism ban.

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

RIKILT serves as scientific officer and qq. as board member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are members 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 on behalf of the board organises the annual IAG proficiency test on animal proteins in feed, 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.

A cooperation was started with the NRLs of France and Poland for the development of monitoring methods for hydrolysed proteins in the framework of a research project in the WOT programme.

10 NRL Feed Additives and national evaluation of dossiers / advice

Coordinator: Jacob de Jong

10.1 Activities within the EURL–NRL network

10.1.1 Workshop

RIKILT Wageningen University & Research is the Dutch NRL both for the Regulation on Feed Additives Authorization (Regulation (EC) No 1831/2003) as well as for Feed Additives Control (Regulation (EC) No 882/2004 and its successor Regulation (EU) 2017/625). The Dutch NRL participated in both workshops, organised by the corresponding EURLs (JRC-Geel, Belgium).

Feed Additives Authorization
The annual workshop for Feed Additives Authorization was held from 17-18 October 2018 in Brussels.
A representative from the European Commission gave an update about a vitamin B2 product that proved to contain a genetically modified *Bacillus subtilis* production strain. Finally, after a negative EFSA opinion, the existing authorization was withdrawn.
In a contribution from FEFANA, an overview was given of industry experience on analytical aspects of feed additives. Among others the potential problems with the applicability of the existing methods for the enumeration of yeasts for coated formulations were addressed.
In a presentation by the EURL, the status of methods for botanically defined flavourings was explained. In the dossier evaluation, the EURL will focus on the methods proposed for the determination of the phytochemical markers - active substances in the range proposed. This is only possible in feed additives. The EURL has published a technical guide for applicants in order to facilitate their work.
A representative from EFSA reported about the risk assessment for chemically and botanically defined flavourings, specifically focusing on prospects and concerns of two safety assessment approaches, viz. the whole mixture and component based approach. He informed the consortium about a public consultation by EFSA regarding draft harmonised methodologies for risk assessment of combined exposure to multiple chemicals (MIXTOX).

Feed Additives Control
The annual workshop for Feed Additives Control was held from 18-19 October 2018 in Brussels.
The results of the proficiency test for total selenium in compound feed for rabbits were reported by the EURL (see below for the results of the Dutch NRL). The EURL also organised a training for a multi-method for carotenoids. The Dutch NRL participated in this training.
The state-of-play of the CEN-projects for standardization of multi-methods for (i) vitamins A, D and E and (ii) carotenoids was presented by the respective project leaders.
A representative from the European Commission gave an update about the review of Regulation (EC) No 152/2009, containing the official methods of sampling and analysis for feed. A first meeting of a working group has been organised by DG-SANTE in August 2018 to discuss proposals from member states and EURLs. No decisions have been taken yet. Further meetings will be organised in 2019, eventually leading to a revision of Regulation (EC) No 152/2009.
In a Topic Discussion with contributions from several participants the state-of-play of the research on the reliability of the official method of analysis for urea, based on spectrophotometry (Reg. (EC) No 152/2009) was discussed. A distinction should be made between the legal use of urea as a feed additive in ruminant feed, with contents that are mostly in the range of 1 – 8% and the illegal use of urea as adulterant in e.g. pet food and yeast. A representative of the Latvian Institute of Food Safety, Animal Health and Environment, BIOR laboratory informed the consortium about a comparison of LC-MS/MS and the official EU-method for the determination of urea (0.002 – 0.005%) in pet food. The results obtained with the official EU-method were much too high. The Dutch NRL informed the consortium that, in a collaboration with Chemisches und Veterinäruntersuchungsamt Westfalen, a

comparison was made for ruminant feeds between the official EU-method and a method based on LC-MS/MS. For products containing relatively low contents of urea (label values of 0,1 - 0.5%), the official method leads to an overestimation while for higher contents comparable results are obtained. The EURL will perform further experimental work to establish the applicability of the various methods for control of urea as a feed additive and as an adulterant.

10.1.2 Dossier evaluation on request of the EURL for Feed Additives Authorization

In 2018 the NRL acted as the rapporteur laboratory by drafting an initial evaluation report about the methods of analysis submitted by the applicant for a preparation of four bacteriophages under the category / functional group 4(d) 'zootechnical additives' / 'other zootechnical additives'. Meanwhile the evaluation report has been published by the EURL
Furthermore the NRL commented on 30 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: nutritional additives (amino acids and trace elements), sensory additives (flavourings and colourants), technological additives (anti-caking agents) and zootechnical additives (digestibility enhancers and other zootechnical additives).

10.1.3 Participation in proficiency tests

The EURL for Feed Additives Control has organised a proficiency test (PT) for total selenium. RIKILT, as NRL for Feed Additives Control, participated and 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.

The NRL participated in two PTs organised by another laboratory in the Netherlands.
The first PT consisted of a milk powder and a pig feed containing coccidiostats. All compounds were detected and quantified with sufficient z-scores between -2 and 2.
The second PT consisted of a laying mash and an infant milk powder sample containing coccidiostats. For the laying mash the NRL analysed all components with sufficient z-scores between -2 and 2. For the infant milkpowder sample the number of participants was too low to perform statistical analysis.

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 2018 25 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 regarding the mixing of the additive in feed and possible risks related to cross-contamination to other feeds. The requests concerned among others probiotics, enzymes, pigments and zootechnical additives.
For 24 national requests it was evaluated if it concerned Genetically Modified Organisms (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 2018 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 on the suitability of the official EU-method for urea in feed in preparation of a meeting at EU-level. The Netherlands Food and Consumer Product Safety Authority (NVWA) was advised on the availability of methods that allow the determination of inert iron by laboratory analysis and on the validity of a statement of a feed producer regarding the measurement uncertainty for zinc in compound feed.

11 National Reference Laboratory for GM food and feed

Coordinator: Ingrid Scholtens

11.1 Activities within the EURL – NRL network

11.1.1 Participation in EURL-NRL workshops

RIKILT participated in the Steering Committee meetings in February and June 2018 (Ispra, Italy). At these meetings the 14th NRL and the annual 28th Plenary meeting were prepared (2-4 October 2018). Participations in the Steering Committee meetings and the Plenary meeting were financed as part of the GMO method validation project WOT-02-004-005 ('Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen').

A Training Workshop on GMO screening Strategies was organised by the EURL GMFF, CRAW and RIKILT, 23-25 May, Gembloux, Belgium. The programme included 2.5 days of presentations, interaction and discussion. The goal was to exchange experiences and practices towards a harmonisation of the GMO screening. It was agreed that coverage of number of GMOs is considered important in screening but it should not be the main aim. The preferred amount of coverage is also highly dependent on the research question. It was also suggested to formulate minimal coverage rules or a minimal screening set. There is a wish for harmonisation of GMO screening and a need for guidance and harmonisation, but not for standardisation. Flexibility should be allowed to fit future developments. Participation in 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').

The 14th EURL-NRL meeting was attended on 2nd October 2018 in Ispra, Italy. At the NRL meeting an overview was given of the proficiency test results of 2018. A difference with earlier PT rounds was that the assigned value was calculated by using the results of two expert laboratories (JRC Geel and JRC Ispra) and not of the participating laboratories. In 2018 one of the samples was a maize bread with highly degraded DNA containing MON810 maize and MON89034 maize. The MON810 maize was hard to quantify in maize bread and several laboratories including the Dutch NRL had not sent in quantitative results due to very low GM copy numbers. The EURL will investigate this problem further. The results of a survey on problems with certified reference materials were presented. Some of the AOCS materials are not ground properly and inhomogeneous. Also there was a demand for certified 1% and 0.1% reference materials instead of having to prepare these percentages by mixing DNA, resulting in non-certified materials.
A third edition of the ENGL measurement uncertainty document is in development. Some changes include addition of Regulation (EC) No 619/2011, streamlining the specific situation of Official Control Laboratories in the EU, digital PCR and a new approach to measure the uncertainty with real samples. The last part of the meeting consisted of a tour de table where every member state could highlight some aspects of their NRL laboratory. The Dutch NRL have mentioned that the Dutch board of Accreditation, 'RvA', requires participation in proficiency test for all matrices under accreditation. The EURL GMFF will try to organise a PT for cotton because until now there has not been a PT for cotton GMO. Also the Dutch NRL mentioned problems with the SYBRGreen rice method. The method can give false positive results with rice and the cry1AbAc method. Sequencing revealed that the signals resulted from endogenous rice sequence. The EURL will follow up on that.

11.1.2 Participation in working groups

RIKILT continued participation in the Working Group on DNA extraction methods and the Working Group on multiplex PCR. The Working Groups were financed as part of the GMO method validation

project WOT-02-004-005 ('Validatie en accreditatie van detectiemethoden voor genetisch gemodificeerde organismen'). RIKILT also participates in the WG on ENGL Procedures. The goal of this Working Group is to review the ENGL procedures and propose modifications where needed.

11.1.3 Participation in proficiency and comparative tests

It is important for the NRL to have enough knowledge and experience to perform the NRL tasks. Also the NRL needs to be accredited for all necessary screening and event-specific methods. Participation in proficiency testing is a requirement for accreditation and participation in the proficiency tests organised by the EURL GMFF is mandatory for the official laboratories (Regulation (EC) No 882/2004). 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. RIKILT participated in a proficiency test for maize bread (MON89034 and MON810) and soybean flower (68416) with good qualitative results.

11.2 Assistance to official laboratories

11.2.1 Quality control

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

11.2.2 Advice

In 2018, the NRL tested a number of Chinese rice samples that were found positive by the OL. The Cry1AbAc element was positive in the SYBRGreen rice method. The positive Cry1AbAc results of the SYBRGReen rice method were confirmed by the NRL but additional sequencing revealed that the sequences were endogenous rice sequences. Therefore the samples were considered to be negative. The NRL has discussed this with the EURL GMFF and proposed to confirm the sequence of positive Cry1AbAc SYBRGreen signals to avoid false positives.

11.3 Scientific and technical support to the competent authority

In 2018 a more risk-based sampling strategy was used for the Dutch GMO monitoring program. The GMonitor module, developed by RIKILT in 2015 was 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. Also information was shared on the EUginius database, developed by RIKILT and BVL (Bundesamt für Verbrauchersschutz 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 in Ispra, Italy and the screening workshop in Gembloux, Belgium. In 2017 RIKILT became the NRL GM food and feed for Ireland. In 2018 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.

12 National Reference Laboratory for milk and milk products

Coordinator: Martin Alewijn

12.1 Activities within the EURL–NRL network

12.1.1 Participation in EURL-NRL workshops

As of 31 December 2017, the EURL milk and milk products was delisted from the list of EURL in Regulation (EC) No 882/2004. With that decision, DG SANCO followed the French initiative to close the EURL, 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 2 parameters in the current EURL scope, and (iv) that priority should be given to a new EURL on plant health. It was stressed that member states can maintain their NRLs.
Without a formal EURL, no EURL-NRL workshops were organised in 2018. However, several member states have expressed the intention to keep their NRL MMP active, and those NRLs share the believe that the current state of (analytical) harmonisation across Europe will gradually be lost without further interaction and cooperation. After an initiative (and future financial support) of the Czech NRL MMP, the Dutch NRL cooperated by approaching the former NRLs across the EU for their willingness to form a voluntary cooperative network of NRLs MMP, to replace parts of the former EURL activities. The Dutch NRL drafted a questionnaire to establish the specific needs and the fitting forms of cooperation across the NRLs in Europe. After revision by the Czech and German NRLs, this questionnaire was sent out, and was returned by 14 NRLs that expressed interest in further cooperation. As a result, the Czech NRL was able to organise and to announce the first international workshop of NRLs MMP, to be held in the Czech Republic, 26-28 March 2019.

12.1.2 Participation in proficiency and comparative tests

No EURL-PTs were organised in 2018, but to keep the quality of the methods up to date, the NRL participated in a number of international proficiency tests:

- PT on somatic cell count (stabilised milk): Test provider ALP (Switzerland). January, May, September, 4 samples per round. Result: z-scores between -0.5 and +0.8.
- PT on somatic cell count (raw milk): Test provider Cecalait (France). March, June, September, December, 10 samples per round. Result: z-score ca -5.
- PT on somatic alkaline phosphatase (stabilised milk): Test provider LGC (UK). January, May, November, 2 samples per round. Result: z-scores between -0.3 and 1.0.
- PT on total flora (raw milk): Test provider Cecalait (France). January, June, September, 10 samples per round. Result: z-scores between 0.3 and 2.5.

The PT-provider for somatic cell account calculates z-scores by taking together results of laboratories using routine and those using reference methods. According to the Dutch NRL this makes the z-scores of this PT unreliable. This PT is therefore only used to compare results of the NRL frequently with that of the OL. For total floral the agreement is that only z-scores > or < 3 are insufficient.

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2018, the NRL provided assistance to the Official Laboratory (OL) by providing reference results on the reference material prepared by the OL, which is used to calibrate the routine equipment at the OL. This year, 12 series with 6 samples raw milk each, were analysed independently by two different technicians using the reference method. Twice, the NRL made a series with raw milk with a number of ALP (alkaline phosphatase) levels for comparative testing between NRL and OL. Both for alkaline phosphatase and total flora it was arranged that NRL and OL participate in the same series of Cecalait PTs (paragraph above), and thus the results on the same material could be used for interlaboratory comparison. The NRL assisted MUVA (Germany) by providing reference results on 3 milk samples in the EPQS 722 proficiency test which aims to generate material with a known reference value for alkaline phosphatase.

12.2.2 Advice

In 2018 the NRL had two meetings with the NVWA where the possibilities for official control on official routine analyses in dairy products were discussed. With the new Control Regulation (EU) 2017/625 coming into force, the current scope of NRL quality control on the dairy routine laboratories needs to be reviewed, and possibly expanded. It became clear that the competent authority has a wide range of analysis performed in routine laboratory, and the NRL suggested a prioritization for the implementation of more formal NRL-like quality control. Decisions on final implementations are expected in 2019.
On request of the CA and the OL, the NRL has studied the trend of the differences between NRL and OL in somatic cell counts for several comparative study schemes since approximately 2011. There is a need to carefully discuss these results, and a meeting between NRL and OL to determine the most appropriate next steps is planned for early 2019.

13 National Reference Laboratory moisture in poultry meat

Coordinator: Erika Silletti

13.1 Activities within the EURL-NRL network

The NRL water content in poultry meat has taken part in two EURL-NRL expert meetings in 2018 together with the other European NRLs and the responsible representatives from Brussels.

13.1.1 Participation in EURL-NRL workshops

Brussels
The first expert meeting took place on March 16th 2018 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. Two topics were added under AOB: '*poultry meat marketed as glazed chicken legs*' and '*water content in packed frozen chicken breast*'.

Presentation 2017 Annual report presented to the CMO Committee in December 2017
Alexander Bernreuther (DG JRC) showed his presentation previously given at the CMO Committee – sector animal products in December 2017. This presentation was based on JRC Technical Report entitled '2017 Annual Report of the Board of Experts in Monitoring Water Content in Poultry meat following Regulation (EC) No 543/2008'.

Short report about the Special Expert Group Meeting 2017 in Ploieşti, Romania
Cristina Dumitrescu from the Institute for Hygiene and Veterinary Public Health – IHVPH (Romanian NRL) presented an overview of the activities at the Special Expert Group Meeting '*Monitoring of Water content in Poultry meat'* held on 10-11 October 2017 in Ploieşti, Romania.

Overview on control data by NRLs 2016 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 poultry meat to the Commission. The overview on the control data obtained by the NRLs for 2016 was presented by Alexander Bernreuther (DG JRC). Detailed statistical analyses and discussion on observations will be available in the '*2017 Annual Report of the Board of Experts in Monitoring Water Content in Poultry meat following Regulation (EC) No 543/2008'*. Furthermore, in addition to the discussed results the following two remarks were added to the discussed issues. Firstly, in relation to the W/P limit, Martin Szentivany (DG AGRI) underpinned that cautiousness need to be used when stating that the current W/P limits are inappropriate as approximately one third of the over-the-limit samples are imports from non-EU countries. At the present moment it is not clear whether the over-the-limit samples are the outcome of changes in poultry breeding and/or processing conditions or if they represent true adulteration cases.

Follow up of the study on the impact of sample homogenisation on the water content in poultry meat and proposals for new ring tests
Yannick Weesepoel (RIKILT, NL) proposed different scenarios as possible follow-up of the proficiency testing as well as of the study on 'Impact of sample homogenisation on the water content in poultry meat'. As follow-up of the proficiency testing (PT), it was proposed that PTs should be provided on a regular base, e.g. every two years and they should be made available to NRLs, but also to field laboratories. One NRL could provide the samples and would evaluate all results. Two types of reference samples were considered to be sufficient to cover the typical range of poultry meat matrices (e.g. chicken breast fillet as well as bone and skin containing chicken meat). In addition, a PT sample from FAPAS (or different supplier) or a certified reference material should also be included. Duplicate

results per materials for moisture and protein content were considered as sufficient. As in-depth knowledge of variables influencing moisture and protein contents due to sample homogenisation is still required, a follow-up of the sample homogenisation study which could be assessed by two different experimental designs was presented. According to the first presented scenario, individual NRLs could investigate a single variable (e.g. sample temperature; use of liquid nitrogen and/or dry ice; different types of homogenisation equipment; environmental humidity; homogenisation time; etc.). A second possible scenario includes the following: Similar to the previous study, one NRL provides samples (two types only, e.g. chicken breast fillet and drumsticks). NRLs homogenise samples with their routine procedure and routine equipment. Homogenised samples (aliquots) will be sent to one NRL for moisture and protein content measurements. NRLs may also measure these samples, which would allow to directly check for any potential systematic bias. The German NRL expressed its interest to test the impact of the use of liquid nitrogen during sample homogenisation on the moisture content of the homogenised samples. The results of this study will possibly be presented during the next meeting this year or eventually in another Expert Group Meeting. Furthermore, Yannick Weesepoel asked the experts in which scientific journal the results of the PT and of the sample homogenisation study could be published. Initially, it was foreseen to publish it in the journal '*Poultry Science'*, but it seems that the editor is not accepting such a manuscript. As alternative the journal '*Meat Science*' was proposed.

Issues related to interpretation of Regulation (EC) No 543/2008 (e.g. 'unknown chilling method', 'measurement uncertainty', 'application of limits for fresh carcases and cuts')
In some areas of controls for monitoring water content in poultry meat, the current legal text of Regulation (EC) No 543/2008 may lead to differing interpretations across Member States. Therefore, a survey undertaken by the European Commission among NRLs prior to the meeting was aimed to explore current common practices used by Member States. The results of this survey will serve to the Commission services purely for information purposes, but it may feed, at a later stage, into clarifications of Regulation (EC) No 543/2008. Alexander Bernreuther summarised the replies of the survey '*Explore current common practices used by Member States*'. Four specific questions were sent on 22 February 2018 to the NRLs. The reply rate was good (~ 68%). Valuable feedback was provided by 19 NRLs (BE, BG, CZ, DK, DE, IE, FR, HR, IT, LV, LT, NL, PL, RO, SI, SK, FI, SE, UK), while 9 NRLs did not provide any answer (EE, GR, ES, CY, LU, HU, MT, AT, PT).

AOB
Two issues were brought up by the participants. The German NRL (Gisela Hahn) reported again on the appearance of a new type of poultry meat marketed as '*iced'* (or glazed) chicken legs from Denmark. It was also asked, whether it is known that this product is also marketed in Denmark. The Danish NRL promised to provide more information. An enquiry from the Czech NRL dealt with the '*water content in packed frozen chicken breast'*. How to cope with the requirements of Regulation (EC) No 543/2008 (Annex VIII; 5.1), where five cuts have to be taken for analysis of the water and the protein content, while in the marketed samples, four frozen cuts are packed together in one package. The experts present were asked for their opinion. There was a common understanding that there is no need to take five packs (= 20 cuts). As splitting of such packs into individual fillets is cumbersome and may lead to biased results of the chemical analysis, it was recommended to take two complete packs (= 8 cuts) to comply with Regulation (EC) No 543/2008.
Finally, Martin Szentivany announced an intention of the Commission to undertake a mini-survey among the NRLs on the implementation of controls in the area of water content in poultry meat in individual Member States.

Dublin, Ireland
The fall meeting of experts was held in Ireland on 17th and 18th October 2018 and was hosted by Teagasc. 'The agriculture and Food Development Authority' in Ireland. Two excursions were planned on 17th October. The first one was a visit to a broiler rearing farm in Shercock, Co. Cavan and the second one was a visit of the Manor Farm poultry processing plant in Shercock, Co. Cavan. The modern broiler rearing farm in Shercock, is one of some 170 farms supplying birds to the Manor Farm slaughterhouse and processing plant. The farm is part of a vertically integrated chain receiving day old chicks, feed and advisory services from the chain. Chicken rearing is intensive in barns. Chickens are raised for 32 days, reaching approx. 2.2 kg of live weight at slaughter. Full traceability in terms of birds reared and feed consumed is ensured while strict biosecurity measures are applied at the farm.

As second excursion, the largest poultry slaughterhouse and processing plant in Republic of Ireland, Manor Farm in Shercock was visited. The Manor farm, formerly family owned by Conor family was taken over in 2017 by Swedish group Scandi Standard. The company owns its own feed mills, two own hatcheries and is supplied from by some 170 farms from the neighbouring counties. It slaughters almost 1 million birds per week, reaching an annual production capacity of some 200 000t. The company's strategy is meeting a growing domestic demand for poultry meat (predominantly breast), while a part of frozen production is exported to France and UK and the other part to third countries, such as South Africa.

The Expert group meeting was held on the 18th October. Two presentations were given by Ciara McDonnell and Colm OBric. Ciara McDonnell provided an extensive introduction of the institute and illustrated the methods and equipment available as well as the meat research programme at Teagasc. Colm OBric from the department of Agriculture, Food and the Marine, DAFM in Dublin provided an overview of the poultry industry in Ireland. In Ireland there are 51 rearing sites and 155 broiler breeder sites. There are 4 hatcheries, but about 95% of all Irish broilers are born in 2 hatcheries. The broiler industry is very integrated using harmonised standards. The downside of this is a massive potential for losses due to zoonotic diseases (avian influenza, Newcastle's disease, etc.). All Irish poultry meat processors use air chilling and almost all poultry meat products are sold as fresh rather than frozen (1/3 as carcases and 2/3 as cuts). There is a substantial market for fresh chicken fillets. Legs, wings and offal are exported to South Africa and France.

These presentations were followed by that one of Alexander Bernreuther (JRC-Geel) evaluating and interpreting the national control data from 2017.

Evaluation and interpretation of national control data from 2017 by Alexander Bernreuther (JRC-Geel)

Alexander Bernreuther (JRC-Geel) presented the first assessment of the 2017 control data. A detailed evaluation will be included in the 2018 Annual Report, which will be distributed among the NRLs at the beginning of 2019, as well as at the next Annual Expert Group Meeting in Brussels in March 2019. Among the results, presented by Alexander Bernreuther, it is worth to mention the following findings:

- The introduction of new fields in the ISAMM reporting system offered additional possibilities for an in-depth evaluation of national control data. With the exception of 2 NRLs, who reported their control data before the implementation of the ISAMM modifications, data for the new fields (e.g. source of sample, country of origin, temperature condition of sample, counter analysis) were mostly provided.
- There is a continuation of the trend from the last years towards improved consistency and completeness of reported data sets.
- Compared with 2016 and 2015, there was a continuation of the trend towards the increased use of air spray chilling for carcases, while the use of immersion chilling decreased. The number of data sets indicating air chilling remained stable (~13%), while in only 0.7% of all data sets 'chilling method unknown' was indicated, which is a clear improvement compared to previous years (4% in 2016 and 2015).
- For poultry cuts, the upwards trend of reporting 'chilling method unknown' was stopped, but the percentage is still high (~38%). As in previous years, this is mainly due to imported chicken meat from non-EU countries, where the chilling method applied could not be established as the labelling of chilling methods is not mandatory. While the percentages of air chilling and air spray chilling remained unchanged, a slight increase of reported data sets using immersion chilling was noted (14% vs 11% in 2016).
- Similar to previous years, in 2017 about 47% of all cuts were classified as 'chicken breast fillet; without skin', about 40% as 'chicken thighs, drumsticks, legs, legs with a portion of the back, leg quarters, with skin' and 6.5% as 'chicken breast; with skin', which is a significant increase compared to 2016 (2.5%). The rest (about 6%) were turkey cuts, classified in three different categories (no data were reported for 'turkey breast, with skin').
- About 21% of all data sets for chicken cuts were over-the-limit, which is less than in 2016 (about 25%). One has to keep in mind that when splitting the data into EU and non-EU, about 14% of the EU cuts were over the limit (21% in 2016), while about 46% of the non-EU cuts exceeded the limits

(41% in 2016). An increase was observed regarding turkey cuts being over the limit (11% in 2017, 7% in 2016 and 3% in 2015). None of the turkey cuts were imported from non-EU countries.
- In 50% of the over-the-limit cases for EU samples, the limit was only slightly exceeded (< 0.10), while approx. 64% of the non-EU samples exceeded the W/P limit only slightly (< 0.10). Still, there is a rather constant percentage of cases being largely over the limit (> 0.20) over the last four years for all reported cuts.

Round table discussion on implemented guidelines and modifications of the ISAMM reporting tool for national control data

The experts shared their first experiences with the implemented modifications of the ISAMM reporting tool. In principle, there were no problems, but in certain cases the requested information is not available, thus could not be reported. Several MSs reported that too many samples are being found close or above the limit, which seems to be mainly due to reduced chicken slaughter age, breeds and feed. There is a need to cope with the current situation of modern poultry rearing, which should not be compared with the situation 1990s, when the current legal limits where established. Indeed, a broader consensus emerged among the present NRLs that this needs to be reflected in the future revision of the applicable legal limits of water content in poultry meat.
Furthermore, eight NRLs stressed the fact that there is a gap in Regulation (EC) No 543/2008, i.e. the Regulation does not clearly state that measurement uncertainties deriving from the water and protein content determination in the laboratories are already accounted for in the fixed water/protein limits.

EU poultry market

Update on the current situation on EU poultry market by Martin Szentivany. The experts were updated on the current market situation on the EU poultry meat market and a short-term market outlook with particular emphasis on poultry meat imports from third countries.

AOB

Martin Szentivany summarised the feedback of the recent 'Survey on national control systems in relation to the checks of water content in poultry meat under Commission Regulation (EC) 543/2008'. The key objective of the survey was to gather basic information on the organisation of control systems across Member States and contribute to information sharing within the Expert Group. After validating survey replies with NRLs, its summary will be distributed within the Expert group.

13.1.2 Organisation and participation in proficiency and comparative tests

A proficiency test, organised by RIKILT, was held for moisture and protein content in poultry meat between May and September 2018. This second proficiency test followed the previous one hold in 2016, which was also organised by RIKILT (Yannick Weesepoel). Erika Silletti (RIKILT, NL) presented the first results of the 2018 proficiency test on chicken breast fillets and drumsticks. At the first glance, the results are very promising; only in few cases questionable z-scores were found. Nonetheless, there is an open issue with the setting of the so-called target standard deviation, which is used to assess homogeneity and stability of the PT samples as well as of the results of the participants. As the concept of using reference values cannot be applied (all NRLs are equally seen as reference laboratories), the consensus value concept was applied. But by doing so, target standard deviations need to be defined beforehand, e.g. based on previous PTs and experience with similar materials. This issue needs to be tackled before the finalisation of the report.

13.2 Assistance to official laboratories

13.2.1 Quality control

RIKILT has organised two quality control rounds (March 2018 and September 2018) in order to ensure that the official laboratory and RIKILT obtain statistically similar results for moisture and protein analysis in poultry meat. The quality controls comprised an inter laboratory check of the moisture and protein analysis conducted on homogenised reference samples of chicken fillet meat and chicken legs.

13.2.2 Advice

The results of both rounds resulted in the observation that the quality of the moisture and protein analysis of the reference samples were acceptably reproducible on an inter laboratory basis. 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 practices.

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 were the NRL experts were consulted.

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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.