National Reference Laboratories Wageningen Food Safety Research

Annual report 2019

M.Y. Noordam, C. Dirks, J.G.J. Mol, G.M.H. Brust, L.L. Leenders, A. Gerssen, J.J.P. Lasaroms, L.W.D. van Raamsdonk, J. de Jong, I.M.J. Scholtens, M. Alewijn, E. Silletti

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Wageningen, December 2020

WFSR report 2020.019

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Contents

Preface

Wageningen Food Safety Research (WFSR) 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 WFSR'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, WFSR reports on the execution of its NRL tasks in 2019.

Summary

National Reference Laboratories (NRLs) are part of the system responsible for the control and enforcement of EU food and feed law. Wageningen Food Safety Research (WFSR) 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 WFSR's NRLs in 2019. These NRLs are for: halogenated persistent organic pollutants in food and feed, pesticides in products of animal origin, mycotoxins and plant toxins in food and feed, metals and nitrogenous substances in feed and food, processing contaminants, 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, additives for use in animal nutrition (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 2019, 15 workshops have been attended by NRLs of WFSR (former name, before June 2019, RIKILT Wageningen University & Research). 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). Wageningen Food Safety Research (former name, before June 2019, 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 December and June 2019.

These subjects are:
- Halogenated persistent organic pollutants in feed and food
- Pesticides in products of animal origin and commodities with a high fat content
- Mycotoxins and plant toxins in feed and food
- Metals and nitrogenous substances in feed and food
- 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 products' an EURL was deemed not to be required anymore as of January 1, 2018. The Dutch ministries decided however to keep a NRL for 'Milk and milk products'.

The objective of this report is to give an overview of activities performed by WFSR's NRLs in 2019.

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 in 2019 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) was 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 ministers of LNV and VWS are the designated CA, these ministers mandated the Food and Product Safety Authority (NVWA) to perform the tasks of a CA. The mandated CA is responsible for designating laboratories for the analysis of official samples, samples taken for official control purposes. In addition, the mandated CA is responsible for making the multiannual 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 WFSR.

Table 1.1 *List of EURLs relevant for NRLs WFSR*

Substances/product group	EURL
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 and plant toxins in feed and food	Wageningen Food Safety Research, Wageningen, The Netherlands
Metals and nitrogenous compounds in feed and food	National Food Institute, Technical Institute of Denmark, Copenhagen, Denmark
Processing contaminants ((including PAHs)	National Food Institute, Technical Institute of Denmark, Copenhagen, Denmark
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)	Wageningen Food Safety Research, Wageningen, The Netherlands
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

Substances/product group	EURL
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. There are three EURLs for the different compound groups in Directive 96/23/EC, in the Netherlands there is only one NRL for all compound groups.

** 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 are responsible for (Regulation (EU) 2017/625, article 94(2)) the following tasks insofar they are included in their annual work programmes:

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

WFSR 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 2019 has been drafted in 2018. In addition, budgets for personnel, and facility and equipment costs have been drawn up. The working plans for 2019 have been positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of LNV and the Ministry of VWS) and have been approved by LNV. Working plans are based on NRL tasks as described in Regulation (EU) 2017/625.

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 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. The designation was updated in December (LNV) and June (VWS) 2019 to take into account the change of the name of the institute into WFSR. 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 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 (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 which are used to test official samples should be validated and included in the laboratory's accreditation (Article 37 of Regulation 2017/625). 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	For contaminant/residues/products
Regulation (EU) 2017/644	• Dioxins, dioxin-like and non-dioxin-like PCBs
SANTE/11813/2017 *(as of January 1, 2020 SANTE/12682/2019)*	• 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 (*as of December 14, 2019: Regulation (EU) 2019/627*)	• 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) 152/2009	• All parameters in feed (a.o. GMO, animal proteins, feed additives, contaminants)

2 National Reference Laboratory halogenated persistent organic pollutants (POPs)

Coordinator: Caroline Dirks

2.1 Activities within the EURL-NRL network

2.1.1 Participation in EURL-NRL workshops

In 2019 two EURL workshops have been held, one in Riga (Latvia, BIOR) and one in Freiburg (Germany, CVUA).

At the first workshop (Latvia, 14-15 May) Frans Verstraete, the representative of the Directorate-General for Health and Food Safety (DG SANTE) of the European Commission gave an update on regulatory issues in regard to POPs in feed and food at EU level. Furthermore he gave a summary of RASFF-notifications related to dioxins, dioxin-like PCBs (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs) since the last EURL/NRL meeting in October 2018. Four RASFF-notifications were related to feed and three to food. In response to a request from the Commission, the EURL published a position paper about options for lowering legal maximum limits (MLs) for dioxins considering the analytical point of view and options for changes of analytical requirements. This position paper was discussed.

At the second workshop (Freiburg, 26-27 November) DG SANTE representative Frans Verstraete gave an update on future revisions of the regulation on MLs for dioxins and dl-PCBs. Possibly the dioxin Toxic Equivalence Factors (TEF) values that are used to calculate the sum of dioxins and dl-PCBs (expressed in toxic equivalents (TEQs)) will be revised and for the matrix horsemeat a ML will be established. A public consultation on this new regulation will probably be held early 2020, the new regulation will be published in 2021. Thorsten Bernsmann (CVUA) presented a case-study from Ennepetal (Germany) where an incident occurred in a silicon factory. During this incident large amounts of PCB 47 were released into the surrounding environment. The highest levels were detected in grass, around 5% higher than what was known from the monitoring the CVUA had been performing since the ‘90s in this area. However usually PCB 47 is not monitored since it is not an indicator PCB. The question rose, if PCB 47 should be included in the list of indicator PCBs (as listed in Regulation (EC) no 1881/2006) and if the metabolism pf PCB 47 is comparable to that of PCB 52 which is an indicator PCB. From EFSA Martin Rose furthermore informed the NRLs on the state of play for dioxins, brominated flame retardants (BFRs), chlorinated paraffins (CPs) and polyfluoroalkyl substances (PFAS):

- For dioxins a tolerable weekly intake (TWI) of 2 pg TEQ/kg body weight was established by EFSA in 2018 for dioxins and dioxin-like PCBs.
- On the BFRs, six scientific opinions have been published until now. Unfortunately due to limitations and uncertainties in data on toxicity EFSA could not to derive health based guidance values. Also the human intake was hard to calculate by EFSA due to the limited number of food categories for which concentration data was available. This also applies for PBDEs, TBBPA, brominated phenols and HBCDD – more data is needed on concentrations in specific food groups (e.g. marine origin, food specific for infants and toddlers). Also, information on production and use of BFRs is often missing. A new opinion on HBCDD and possibly also PBDEs will be published in 2020.
- EFSA is currently working on a revision of the drafted opinion on CPs which was endorsed in July 2019 and underwent public consultation. Exposure assessment for CPs could only be performed for short-chain chlorinated paraffins (SCCPs) and medium-chain chlorinated paraffins (MCCPs), there was a lack of data for long-chain chlorinated paraffins (LCCPs). The mean exposure to SCCPs and MCCPs ranges between 1.9 and 35 ng/kg b.w. per day (SCCPs) and 4.9 to 87 ng/kg b.w. per day (MCCPs), respectively. The assessment was performed based on concentration data in fish meat submitted by Germany between 2014 and 2017. It was concluded that risk assessment of CPs is still

difficult due to the lack of toxicokinetic data for humans and a limited number of CPs that have been tested in the available toxicity studies.
- EFSA is also working on a revision of its opinions on PFAS.

The core working group (CWG) 'Dioxin patterns' developed a system for the comparison and possible identification of congener patterns for PCDD/Fs and PCBs based on two pillars – the data base with congener pattern of known contamination sources and a search algorithm using the profiler software developed by Jerry Hart (UK). This database was presented at this second workshop and all participants received a USB stick with the database.

2.1.2 Participation in working groups

The Dutch NRL participated in 3 core working group (CWG) meetings in 2019. There were meetings on PFASs, BFRs and CPs, the latter two were held at WFSR and the CWG on PFASs was held at Örebro University in Sweden.

The CWG meeting on BFR was held on September 24th. Alwyn Fernandes who leads the BFR CWG gave an update on the LOD/LOQ in the new guidance document written by the 4 EURLs for contaminants, 'Guidance document on analytical parameters for the determination of organobromine contaminants in food and feed'. Further the analytical criteria for PBDEs and HBCDD were discussed, for which no regulations are in place yet. The second main topic of the meeting was the occurrence of emerging brominated contaminants. A few presentations were given by the NRL's: Philippe Marchand (LABERCA) talked about emerging BFRs and a shortlist was proposed i.e. brominated dioxins, TBBPA, DBDPE, BTBPE, bromophenols, PBEB, HBB, PBT, PBBz, DBE-DBCH, BEH-TEBP and EH-TBB. The next steps include the completion of the guidance document on analytical parameters, the analytical methods and further discussion on the choice of relevant, emerging brominated contaminants. A training course on the analysis of brominated contaminants with focus on PBDEs and HBCDDs will be held on 03-04 November 2020 in Freiburg. Selected emerging contaminants will be included also in future EURL PTs as additional optional parameters.

The CWG meeting on chlorinated paraffins (CPs) was held on September 25th. It was discussed that while there are currently several different research groups active in the field of CPs, the EFSA Draft Opinion revealed an alarming lack of robust data on CPs in food and feed. The only data available was on fish meat from Germany. This shows again that a general guidance document for the analysis might be necessary to motivate more official laboratories to adapt a method and start analysing samples. Therefore, publishing this guidance document should be the main goal for this Core Working Group in the next year. There is an ongoing search for commercially available CP standards. The EURL will investigate 15 SCCP mixtures, 13 MCCP mixtures and 9 LCCP mixtures from Dr. Ehrenstorfer. The EURL is also coordinating the synthesis of chain length specific standards by the university of Hohenheim in Germany. The availability of standardized chain-length or single congener standards will improve the inter-laboratory reproducibility and will provide the community with reliable data on chlorinated paraffins in food commodities other than fish meat. This will be extremely important in the near-future so that proper risk assessment can be done.

The CWG meeting for PFASs was held on June 18th-19th where the TWIs for PFOA and PFOS published by EFSA in 2018 were discussed. Based on these guidance values and typical consumption habits analytical LOQs for PFOA and PFOS in meat, milk, fish and water were derived by the CWG PFAS. These values were compared with those achieved by NRLs and OLs based on a EURL survey launched by the EURL for halogenated POPs in 2019. With the exception of milk it should be possible to meet the new calculated LOQs for PFOA and PFOS. However, the range of reported LOQs was very broad among the participants. It was mentioned that PFOA, its salts and PFOA-related compounds are now included in annex A of the Stockholm Convention.

2.1.3 Participation in proficiency and comparative tests

The NRL has participated in PTs that were organized by the EURL. In addition the NRL has participated in PTs organized by QUASIMEME (see Table below).

Table 2.1 *List of PTs*

Name of PT	Topic	z-score
QUASIMEME	Brominated Flame Retardants (PBDEs) in bream filet	Between -2.15 and -0.84
QUASIMEME	Brominated Flame Retardants in mussels	Between -0.61 and 0.32
QUASIMEME	Perfluorinated Alkyl Substances (PFASs) in mussels and flatfish dab	Between 0.68 and 2.92
EURL	PCDD/Fs, PCBs, PBDEs and BFRs in grass	Between -1.2 and 1.9
EURL	PCDD/Fs, PCBs, PBDEs and BFRs in egg yolk	Between 0.1 and 1.6
EURL	Chlorinated paraffins in pork sausage	Under evaluation

The majority of the results were satisfactory (z-scores between -2 and +2), however there were a few results that were either < -2 or > 2. The unsatisfactory results were: a PT from QUASIMEME which was analysed for PBDEs in bream filet, 2 out of 11 z-scores were < -2. When the samples were re-analysed with a new calibration curve, the z-score was -1.4 and -0.8 respectively. For PFASs one unsatisfactory result was obtained i.e. z-score 2.92 for total-PFOS. However, the variation between the reported results from the different laboratories was high for this PT which makes the calculation of a z-score unreliable.

2.2 Assistance to official laboratories

2.2.1 Quality control

In two rounds (spring and autumn), 8 dairy fat samples were exchanged with the OL. The results were satisfactory, although a slight negative bias for the PCB-TEQ was observed in the first round. In the second round this bias was absent. Two samples from 2016 and 2017 were exchanged with the OL again in 2019 for repeat analyses, to monitor the performance (reproducibility) over time. The reproducibility for the first round was good and met the EU criteria (as described in Regulation (EU) 2017/644) of <15% for dioxin-TEQ and total-TEQ for both samples included in this study part. However, the RSD_R for dioxin-TEQ and total dioxin-TEQ in the second round was between -63% and 14%, this was due to the low concentrations of dioxins and PCBs in the sample, far below the requirements for limit of quantification in milk. This was reported back to the OL.

2.2.2 Advise

Information about the extension of the scope and renaming of the EURL (and NRL) to halogenated Persistent Organic Pollutants was exchanged with the OL in March 2019.

2.3 Scientific and technical support to the competent authority

The ministries of LNV and VWS, and NVWA and RIVM were supported by the NRL with regards to EFSA opinions on dioxins and PFASs published in 2018, and the draft EFSA opinion on chlorinated paraffins from 2019. There also has been discussions on dioxins and PCBs in fish, on PFASs in fish, and POPs in food and feed with these stakeholders in general.

2.4 Contacts with other NRL's

The NRL contacted the EURL during 2019 to discuss issues with a PBDE-congener that interfered with the analysis. The EURL advised on how to deal with this contaminant in future.

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 combined event by all four pesticide EURLs, in even years separate meetings are held by the individual EURLs.

In 2019 a joint meeting was held on 25-27 September in Copenhagen which was attended by WFSR. WFSR is NRL for pesticides in food and feed (all four EURL domains), and for residues in live animals and animal products. This section deals with the NRL task assigned to WFSR for certain residues in live animals and animal products through Commission Decision 98/536/EC, which relates to the EURL for pesticides in products of animal origin.

The purpose of the workshop was to inform the NRL network about relevant matters from the Commission, to exchange technical information (analytical methods, new technologies, issues with certain pesticide/matrix combinations), to present the set up and discuss the outcome of the annual EU proficiency tests, and to present the EURL program and activities for the next year. Furthermore, the bi-annual revision of the 'Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed' was presented and discussed.

The items presented and discussed are briefly summarized below:

- Gonzalo Granado Lois from the European Commission (DG Health and Food Safety, Directorate F) gave a presentation on audits to member states and third countries performed by Directorate F. Main outcomes of Member State audits were deficiencies in designation of reference and official laboratories, lack of specification of tasks, lack of oversight of the Competent Authorities, effectiveness compromised by low number of samples per lab, and sampling–report time-lag which compromises follow-up enforcement actions. For 2020 audits will focus on sustainable use of pesticides and Third Countries. Future audits will focus more on pesticides that require a dedicated single residue method which often get less attention in monitoring and control programs. The second part of the presentation dealt with the new Official Control Regulation (OCR, Regulation (EU) 2017/625), and the description of roles and tasks of the EURLs, NRLs and OLs.
- Luis Carrasco Cabrera from the Pesticide Residues Unit of EFSA presented on the MRL review and monitoring, covering both pre- and post-marketing assessments. EFSA collaborates with the EURLs mainly on availability of validated analytical methods, LOQ proposals, and availability of analytical standards. The regulations covering approval and renewal or non-approval of active substances (Regulation (EC) no 1107/2009), and on pesticides MRLs (Regulation (EC) no 396/2005) were briefly outlined. The most recent European Union report on pesticide residues in food (2017 data) made by EFSA was presented. Overall, residues were found in 46% of the almost 90,000 samples taken in the EU countries. MRLs were exceeded in 4.1% of the samples. An exposure assessment was performed based on the residue data and food consumption data using the PRIMo model, for the 12 commodities covered in the EU coordinated monitoring program. The acute reference dose (ARfD) was exceeded for 24 pesticides in 197 samples. The highest number of ARfD exceedances occurred for chlorpropham, iprodione, chlorpyrifos, imazalil and thiabendazole, mostly in pears, potatoes, kiwis, oranges and carrots. In response, the approval of the pesticides has not been renewed, and/or the MRLs have been lowered. Assessment of chronic exposure did not reveal any

risks (no exceedances of the ADI), with the exception of dithiocarbamates when using an upper-bound scenario. It was concluded that the probability of European citizens being exposed to pesticide residue levels that could lead to negative health outcomes is low.

- Ioannis Sitaras, convenor of the European Accreditation (EA) technical network for food and feed testing, gave a lecture on the EA guidance on accreditation of pesticide residue analysis in food and feed. The aim here is to provide guidance in development of harmonised accreditation of pesticide residue analyses. Although accreditation bodies from different Member States all audit according to ISO17025, the interpretation and conduct of audits differ to some extent. This is especially true for flexible scope accreditations, an important aspect in pesticide analysis, given the very high number of pesticide/matrix combinations.
- Eight technical presentations were given. Nadja Bucher (BVL, Germany) addressed the issue of analysis of metabolites of pesticides included in the residue definition. Kirsten Halkjaer Lund (Danish Veterinary and Food Administration) and Sadat Nawaz (Fera, UK), gave their view on the challenges and solutions regarding preparing and accuracy of pesticide mix standards for multi-methods. Mette Poulsen presented on the validation of multi-methods specifically for rice-based baby food. Lucie Humbert (SCL, France) shared her experiences with automation of a the QuEChERS extraction/cleanup method, which turned out to take a lot of efforts. Sonia Herrera Lopez (WFSR, Netherlands) presented on tips and troubleshooting of difficult (from analytical point of view) pesticides such as glyphosate and other polar ionic compounds. Friederike Habedank (State office, Mecklenburg Vorpommern) took up the challenge to combine multi-methods for medium and highly polar pesticides in a two-dimensional liquid chromatographic system (2D-LC) coupled to high resolution mass spectrometry (HRMS). Amadeo Fernandez Alba (University of Almeria, Spain) presented an overview of the possibilities of full scan HRMS, the various acquisition modes and the pros and cons associated with them.
- Discussion of EURL-proficiency tests (PTs). In total 5 PT were organised in 2019 by the various EURLs: two on products of animal origin: multi-residue methods (AO14), and SRM-pesticides (SRM14), both in liver (special request of the Commission). For AO14 a target list was provided with 59 mandatory pesticides and another 30 pesticides to be analysed on a voluntary basis. In total 12 and 7, respectively, were present in the material. Levels ranged from 0.04 to 0.25 mg/kg. In total, 100 laboratories (NRLs and OLs, plus several laboratories from third countries) participated. For the mandatory pesticides, the percentage of laboratories reporting these pesticide was 92-95%. For the voluntary pesticides this was lower: 46-73%. The percentage of laboratories with satisfactory performance varied for the different pesticides, from 89-95% (50-90% for voluntary pesticides). As measure for the interlaboratory variability, the robust relative standard deviation was used which varied from 7% to 45%. In most cases it was in the range 13-20%, i.e. below the 25% that is used as fixed value for expanded measurement uncertainty for enforcement purposes.
  For the SRM14, a target list was provided with 1 mandatory pesticide (glyphosate) and another 31 pesticides to be analysed on a voluntary basis, these included highly polar pesticides and certain pesticide metabolites. Determination of all pesticides required multiple SRM-methods. Besides glyphosate, 15 SRM-pesticides turned out to be present in the material. Levels ranged from 0.05 to 0.75 mg/kg. In total, 60 laboratories participated. Glyphosate was measured by 75% of the laboratories. For the other pesticides this ranged from 19-81%. The percentage of laboratories with acceptable performance varied for the different pesticides, from 71-98%. The robust relative standard deviation varied from 12-41%. The results of the Dutch NRL in these PTs are presented in 3.1.3.
- AQC document. A full session was devoted for presentation and discussion of the proposed revision of the analytical quality control (AQC) document: 'Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed' (existing version: SANTE/11813/2017), moderated by Tuija Pihlstrom. Main adjustments were on correction for recovery (presented by Hans Mol of the Dutch NRL), measurement uncertainty (Antonio Valverde), rounding of results (Magnus Jezussek), and an update of the commodity table for feed commodities (Mette Poulsen). After discussion, the modifications were adopted. A new version of the AQC document (SANTE/12682/2019) to be used by 1 January 2020 was issued.
  https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2019-12682.pdf
- Various EURL matters: Hubert Zipper (EURL-SRM) informed on the establishment of a stronger cooperation between OLs in the field of SRM pesticides. Karin Tschiggfrei (EURL-AO) informed the community on a survey of milk-based infant formula that will be performed by the EURLs, at the request of the Commission.

3.1.2 Participation in working groups

WFSR 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 AQC document (see above). Taking into account comments from the NRL-OL network, proposals were made for revision of the guidance document, and these were discussed during meetings in Madrid (31 Jan/1 Feb, 19-20 June) and Copenhagen (25 Sept). During the June meeting also the results of proficiency tests organised by the EURLs in the first half of 2019, were discussed.

3.1.3 Participation in proficiency and comparative tests

The NRL participated in four proficiency tests in the domain of pesticides in products of animal origin. Two were organised by the EURLs, the others by Fapas and the PT-unit of WFSR. An overview is given in Table 3.1. In general performance of the NRL was satisfactory. For four out of 38 pesticide/matrix combinations a questionable result was obtained. For fipronil/sum fipronil in fat the cause was found and the method description adapted to more specifically state that fat needs to be in the liquid state during the entire extraction/cleanup procedure. In the other two cases no cause could be found (note that statistically 5% of all results will fall in the Q-range).

Table 3.1 *Overview of proficiency tests NRL pesticides in products of animal origin*

			Performance 1)		
PT Organiser	**Matrix**	**Analytes**	**S**	**Q**	**U**
WFSR	Egg	Fipronil, Fipronil-sulfone, Sum fipronil	3	0	0
	Chicken fat	Fipronil [2)], Fipronil-sulfone, Sum fipronil [2)]	1	2 [2)]	0
Fapas	Egg	Chlordane, p,p'-DDT, Dieldrin, HCB	4		
EURL-AO	Liver (bovine)	Boscalid, Carbendazim, cis-Chlordane, trans-Chlordane, Chlorpyrifos-ethyl, p,p'-DDE, Deltamethrin, Diazinon, Dieldrin, α-Endosulfan, Flusilazol, HCB, alpha-HCH, beta-HCH, Penflufen, Permethrin, Spinosad, Spinosysn A, Spinosyn D, Tebuconazole	15	0	0
EURL-SRM	Liver (bovine)	Avermectin B1a, Bixafen metabolite (desmethyl bixafen), Boscalid metabolite (M510F017), Bromoxynil, 2,4-DB, DDAC-C10[3)], Fenpropimorph metabolite (carboxylic acid, BF-421-2), Flonicamid metabolite (TFNA-AM), Fluopyram metabolite (fluopyrame-benzamide, M25), Glufosinate metabolite (MPP)[3)], Glyphosate, MCPA, Mepiquat	11	2 [3)]	0

[1)] S= satisfactory, Q = questionable, U = unsatisfactory.

[2)] issue during sample preparation (fat partially solidified).

[3)] no cause could be found.

3.2 Assistance to official laboratories

3.2.1 Quality control

In the Netherlands, besides WFSR, there is one laboratory performing part of the official analyses in dairy products. In the frame of a quality control program, one sample of milk powder (blind sample taken from a previous proficiency test) was sent to the dairy laboratory for determination of organochlorine pesticides. Results were reported to and evaluated by WFSR, and communicated with this OL.

3.2.2 Advise

Feedback was provided to the OL on a new method that is under development in their laboratory.

3.3 Scientific and technical support to the competent authority

Input was provided regarding the fine-tuning of the scope of the National Plan Residues regarding pesticides (acaricides, insecticides, ecto-parasiticides), through the WFSR project leader of that program. Suggestions for an alternative measurement strategy (wide-scope full scan screening rather than more restricted scope quantitative monitoring) were made. It was also suggested to emphasise more on coverage of all relevant animal products and especially eggs in this type of screening.

3.4 Contacts with other NRL's

Regular ad-hoc contacts took place with the other NRLs on pesticides in animal origin (and other commodities) throughout the year. Dissemination and discussion of technical aspects (analysis, legislation, analytical quality control) took place during the international symposia, including: 14th International IUPAC Congress on Crop Protection. Ghent, 19-24 May 2019; 10th International Akademie Fresenius Conference 'Pesticide Residues in Food', Wiesbaden, 27 Jun - 28 Jun 2019; RAFA 2019, Prague. 5-8 November 2019, and the EURL-NRL meetings mentioned under 3.1.1/2.

4 National Reference Laboratory Mycotoxins and Plant Toxins in food and feed

Coordinator: Hans Mol

4.1 Activities within the EURL-NRL network

4.1.1 Participation in EURL-NRL workshops

In 2019 a workshop organized by the EURL for mycotoxins and plant toxins in food and feed (WFSR, Netherlands) was held on 1-2 October in Ede, The Netherlands. Forty representatives of NRLs from EU and EFTA countries, and one Commission representative attended. 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 inform the community on EURL and other activities in the domain of mycotoxins and plant toxins in food and feed.

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

- *News from the Commission, update on legislation, by Frans Verstraete from Directorate-General for Health and Food Safety*. Both in mycotoxin and especially in plant toxins, there are a lot of activities and new regulations under consideration. Mycotoxins. For citrinin, the current maximum level in food supplements based on rice fermented with red yeast (*Monascus purpureus*) will be lowered from 2,000 µg/kg to 100 µg/kg. For other food and feed products no immediate regulatory action seems required. For ergot sclerotia (the kernels) in unprocessed rye, a maximum level of 0.5 g/kg, to be lowered to 0.2 g/kg by 1.7.2022, is proposed. For other unprocessed cereals (except maize and rice), this is 0.2 g/kg. For processed cereal/-products, the ergot alkaloids (substances, sum of six alkaloids plus their epimers), will be regulated, ranging from 100-500 µg/kg. Also here, a transition period applies in some cases with reduced maximum levels to 50-250 µg/kg after 1.7.2022. For cereal-based baby food, a fixed maximum level of 20 µg/kg is proposed. For *Alternaria* toxins, monitoring is recommended with emphasis on alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TeA). For these mycotoxins, indicative levels are proposed for processed tomato products, sunflower seed/oil, and sesame seeds (5-30 µg/kg for AOH and AME, much higher for TeA). For cereal-based baby food this is 2 µg/kg for AOH and AME, 500 µg/kg for TeA. For paprika powder, tree nuts and dried figs, indicative levels are only proposed for TeA (100-10,000 µg/kg). These levels are not food safety levels, but in case of repetitive exceedings, investigations should be performed to find out the cause and possibilities to reduce the contamination. For ochratoxin A, maximum levels are under discussion for several additional, not yet regulated, foodstuffs (dried figs, dried herbs, tea, cocoa powder, sunflower seeds, pistachio nuts, hazelnuts). Discussions on necessity for regulation and the possible maximum levels has been postponed until the EFSA opinion becomes available. For deoxynivalenol (DON) in both food and feed, three other forms will be included (3-acetyl-DON, 15-acetyl-DON, DON-3-glucoside) in the maximum levels. The actual maximum levels for the sum are under discussion. For zearalenon**e**, modified forms (conjugates) are not foreseen to be considered for inclusion in regulation. The same applies to modified forms of fumonisins. Enniatins are under consideration for regulation, but nothing is expected on short notice.
- *Plant toxins*. For erucic acid, adjusted regulated levels are proposed 20 g/kg for vegetables oils, 35-50 for some specific oils and mustard, 4 g/kg fat for baby food. For pyrrolizidine alkaloids (PAs), discussion is on-going on maximum levels in food (herbal infusions (dried product), dried herbs, food supplements) 200-400 µg/kg for sum of PAs (up to 1000 µg/kg for certain herbs). For tea (*Camellia sinensis*) this is 75 µg/kg. Which PAs need to be included in the sum used for legislation is a very complex matter. There are 100s if not 1000s individual PAs. Initially 21 representative

marker PAs have been proposed, later on extended with another three. In the analysis it turns out that a number of other PAs cannot always be separated from the PAs proposed for legislation. This depends on the measurement conditions. To avoid differences in the sum result due to this, a further increase to the sum of 35 PAs is foreseen. For tropane alkaloids (atropine, scopolamine) regulatory levels for baby food will apply to a wider range of ingredients, while maximum levels will also be set for millet, sorghum, maize and buckwheat. Cyanogenic glycosides are currently regulated as hydrocyanic acid (HCN) in apricot kernels, nougat/marzipan, canned stone fruits and alcoholic beverages. Setting of maximum levels for other foodstuffs is being considered (linseed/-products, cassava/-products). For opium alkaloids maximum levels are considered (morphine equivalents = morphine + 0.2x concentration codeine), 20 mg/kg poppy seed, 1.5 mg/kg for bakery products containing poppy seeds. Possible regulation of THC (delta-9-tetrahydrocannabinol) in hemp-derived food is awaiting an EFSA report on exposure. For calystegines (present in potatoes, paprika, aubergines) no risk management measures are needed for the time being and no regulation is foreseen. The Commission requested EFSA opinions on glyco-alkaloids, quinolizidine alkaloids (adopted), and shall be requested for an opinion on grayanotoxins in honey.

- *Six technical presentations were given*. Leo van Raamsdonk (WFSR, Netherlands) and Adél Szabó (NEBIH, Hungary) presented on visual techniques to measure ergot sclerotia in food and feed, and botanical impurities (*Datura, Ricinus, certain Brassica species, Croton tiglium, Fagus silvatica*, etc) in feed. Several presentations with lively discussion dealt with the determination of pyrrolizidine alkaloids. Gerald Ledoux, SCL, (Strassbourg, France) shared his experiences and practical issues with the analysis. Patrick Mulder (WFSR, Netherlands) presented a novel sum parameter approach based on the necine backbone. This method allows for the quantitative determination of the four most important necine moieties, which are obtained after alkaline hydrolysis. The outcome of an investigation by the EURL on the determination of citrinin in red yeast rice capsules was presented by Aleksandrs Versilovskis (WFSR, Netherlands). The issue here is that the capsules need to be included in the analysis which poses challenges with respect to homogenisation of the capsule material. In the end, complete dissolution of the capsule during the extraction procedure was considered a practical solution. He also presented work done by the EURL on method development and validation of *Alternaria* toxins in various matrices (besides tomatoes, also relevant in figs, treenuts, and cereals).
- *Discussion of EURL-proficiency tests (PTs*). Feedback was given on the follow up of last year's PT (Deoxynivalenol (DON), acetyl-DONs, DON-3G). In that PT, many NRLs only analysed for DON. The main reason was that the other DON-forms were not included in legislation and, therefore, got lower priority in method development and inclusion in monitoring, despite the long standing EFSA recommendation to include them. Some NRLs used methods that did not allow inclusion of the other DON forms, and required more time and efforts to change their method. The outcome of a PT (at this stage more considered as an interlaboratory research study) on pyrrolizidine alkaloids (PAs) organised in June-July 2019 was presented. Laboratories used different LC-MS/MS methods for instrumental analysis of the extracts, which different degrees of separation of the PA and PA isomers. It was concluded that full separation of all 35 target PAs was not feasible within one run. It was therefore considered inevitable to include all of them (overlapping or not) in near-future legislation. In Q4 of 2019, the EURL will organise a PT on ergot alkaloids in food and feed.
- *Method performance criteria*. For mycotoxins, method performance criteria for recovery and precision have been described in Regulation (EC) no 401/2006. The criteria vary for the different mycotoxins and levels. An assessment by the EURL revealed that there is no scientific rationale (anymore) for the current criteria, and that they need to be updated. The Commission has asked to EURL to take the initiative and propose new criteria. For this an Analytical Quality Control group with EURL and NRL representatives was established, and prepared a session to discuss proposals for updated or new criteria. A full morning with breakout sessions was held. The outcome will be taken into account in the preparation of a draft revision of the relevant annex in Regulation (EC) no 401/2006. A draft was anticipated to be send to the NRLs by January 2020.
- *Miscellaneous.* To inform the NRL community, an update of activities within CEN for mycotoxins and plant toxins in food (Martien Spanjer, NVWA, Netherlands) and feed (Hans Mol, WFSR, Netherlands) was given. Presentations on developments in EU projects in the area of, or of relevance for, mycotoxins/plant toxins were given by Arjen Gerssen (WFSR) on FoodSmartPhone (fast on-site testing using mobile phone-based devices), and Ine van de Fels-Klerx (WFSR) on the EU project

MyToolBox which aims at integrated management strategies to control and reduce mycotoxins in food and feed.
- EURL working program 2020, will include training activities on analytical methods, and PTs ergot alkaloids (finalisation of PT started in 2019) and on visual methods for ergot sclerotia in cereals.

4.1.2 Participation in working groups

As part of the EURL task, WFSR has initiated a working group on analytical quality control (AQC). A meeting was held on 3rd May in Wageningen in which EURL/Dutch NRL representatives participated. Current procedures and interpretation of legislative performance criteria were exchanged. Based on this preparations were made for a broader discussion at the EURL workshop in October.

4.1.3 Participation in proficiency and comparative tests

The NRL participated in six proficiency tests, four on mycotoxins and two on plant toxins. An overview is given in Table 4.1. In general performance of the NRL was satisfactory. For three out of 32 toxin/matrix combinations a questionable result was obtained. For ergot alkaloids the organiser indicated to use at least 10 g for the analysis. However, the amount of material supplied was insufficient for the routine WFSR procedure (standard addition). For this reason, only 4 g of material could be used for analysis, with risk of higher variability due to sample inhomogeneity. The questionable performance of ergocristinine may have been a result of this. As follow up action, an additional analysis with 10 g sample material using an one-point standard addition was performed, resulting in a satisfactory performance. For future PTs of this kind, more material would need to be requested from the organiser in order to be able to follow the routine in-house procedure and avoid method adaptions for the PT sample only. For fumonisin B1, erroneously the initial result before the required and performed dilution had been reported. This resulted in a questionable performance of fumonisin B1 and also affected the outcome for the sum of fumonisins.

Table 4.1 *Overview of proficiency tests NRL mycotoxins and plant toxins*

			Performance [1)]		
PT Organiser	**Matrix**	**Analytes**	**S**	**Q**	**U**
Mycotoxins					
Fapas	Milk powder	Aflatoxin M1	1	0	0
Fapas	Rye Flour	Ergocornine, Ergocorninine, Ergocristine, Ergocristinine[2)], Ergometrine/Ergonovine, Ergometrinine/Ergonovinine, Ergosine, Ergosinine, Ergotamine, Ergotaminine, α- and β-Ergocryptinine (sum)	10	1[2)]	0
Fapas	Maize	Alfatoxin B1, Dexoynivalenol, Fumonisin B1[3)], Fumonisin B2, Fumonisin (sum)[3)], Ochratoxin A, T2, HT2, Sum T2+HT2, Zearalenone	8	2[3)]	0
Fapas	animal feed	Aflatoxin B1, Dexoynivalenol, Zearalenone	3	0	0
Plant toxins					
PROOF-ACS	chamomile tea	Echimidine N-oxide, Heliotrine, Intermedine N-oxide, Lycopsamine, Trichodesmine	5	0	0
Fapas	Baby cereals	Atropine, Scopolamine	2	0	0

[1)] S= satisfactory, Q = questionable, U = unsatisfactory.

[2)] lower amount than described by organiser had to be used, may have contributed to higher variability due to sample inhomogeneity.

[3)] error in reporting, initial result instead of that obtained after dilution, had been reported.

4.2 Assistance to official laboratories

4.2.1 Quality control

In the Netherlands, since the merger of the NVWA laboratory for food and feed safety with RIKILT in June 2019, only one OL remains. This OL specifically analyses dairy products for presence of aflatoxin M1. For the dairy laboratory, quality control was done by sending standard solutions and milk samples containing aflatoxin M1. Results were reported to and evaluated by the Dutch NRL, and feedback was provided.

4.2.2 Advise

Technical advice was given to the OL following the results of the exchange of milk samples and standards for analysis of aflatoxin M1.

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 WFSR, also the Ministry of Health, Welfare and Sport 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 2019 there was specific exchange of information and interpretation of data regarding hypericine (the presumed active ingredient, not necessarily toxin) in St. John's worth food supplements.

4.4 Contacts with other NRL's

Contacts with other NRLs were through the EURL workshop, through the CEN meetings on mycotoxins in food and mycotoxins/plant toxins in feed, and symposia: 11th conference of the World Mycotoxin Forum, 14-16 Oct 2019, Belfast; RAFA 2019, Prague. 5-8 November 2019.

5 National Reference Laboratory Metals and Nitrogenous Compounds

Coordinator: Hanneke Brust

5.1 Activities within the EURL-NRL network

5.1.1 Participation in EURL-NRL workshops

The EURL for Metals and Nitrogenous compounds is hosted by the DTU in Denmark. The annual EURL workshop was held in Copenhagen on October 23 and 24. The meeting was attended by forty-four participants representing NRLs from each EU member state. The participants were welcomed to the EURL-NRL workshop by the director of the EURL-MN. The workshop started with a session on feed followed by a session on food.

- *Discussion of EURL-proficiency test (PT) in feed*. One of the EURL-PTs concerned the determination of total-arsenic, inorganic arsenic, cadmium, lead, mercury and iodine in seaweed meal. Only a limited number of laboratories participated in the PT for inorganic arsenic (and iodine). Although the determination of inorganic arsenic in food and feed is within the scope of the EURL-NRL network, two thirds of the NRLs do not have an appropriate analytical method. The EURL had therefore organized a training on the determination of inorganic arsenic in feed and food (September 2019) and expects an increase in the number of participants for one of the PTs on inorganic arsenic in 2020. The Dutch NRL did not participate in the training because it already has this expertise. Other topics covered in the session on feed were the determination of the moisture content and future PTs that will be organized by the EURL.
- *News from the Commission*. Frans Verstraete (DG Health and Food Safety) presented recent and future developments of the regulations for heavy metals in feed and food. NRLs were encouraged to monitor metals and iodine in seaweed and products based on seaweed in accordance with Recommendation (EU) 2018/464 and to provide their data to EFSA. Regulation for metals in seaweed is limited. There are concerns about potential health effects due to presence of metals in seaweed. Furthermore, the EU expects to publish a recommendation on monitoring of inorganic arsenic in additional feed materials.
- *Discussion of EURL PTs in food*. The session on food included a discussion on two PTs that were organized by the EURL in 2019. The first PT concerned the determination of cadmium, lead, mercury, nickel and copper in offal (liver). Several laboratories reported unsatisfactory results for nickel. This was attributed to the low nickel level in the sample. Besides the analytical results, the capability of the NRLs to state the compliance with European legislation was discussed. A large number of laboratories did not assess the compliancy for mercury and copper in a correct manner, according to the EURL. Mercury and copper levels are regulated in Regulation (EC) no 396/2005 on maximum levels of pesticide residues in or on food and feed, in contrast to cadmium and lead, which are regulated in Regulation (EC) no 1881/2006 (maximum limits for contaminants in foodstuffs). Since most mercury and copper containing pesticides are nowadays banned, levels in food are a result from the natural occurrence of these elements or from other sources of environmental contamination. Although the EURL stated that several NRLs seemed to be unaware of the regulation on pesticide residues, some NRLs claimed to have assessed the compliancy differently because the source of the mercury and copper levels could not be determined. The second PT concerned the determination of cadmium, lead and nitrate in vegetable based baby food. This was the first PT including the determination of a nitrogenous compound since the scope of the EURL-NRL network was extended with nitrogenous compounds in 2018. The discussion of the PT focussed on limits of determination (LOD) and limits of quantification (LOQ) for metals in baby food. Criteria for the LOD and LOQ are laid down in Regulation (EC) no 333/2007. Because the maximum limits for metals in baby food are low, analytical methods are required with low LODs and LOQs. Several laboratories use methods that do not meet the LOD and LOQ criteria for baby food. For nitrate, over a quarter of

the laboratories reported unacceptable results. A possible reason was expression of the nitrate content as sodium nitrate by some laboratories, whereas the nitrate content should have been expressed as the nitrate ion. The discussion of the PTs was followed by two presentations on the determination of nitrate in food.
- Miscellaneous. The workshop was concluded with an interactive session in which the delegates of the NRLs discussed the determination of detection limits and quantification limits of analytical methods.

5.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to metals or nitrogenous compounds in food and feed in 2019 to participate in.

5.1.3 Participation in proficiency and comparative tests

The NRL has participated in three proficiency tests organized by the EURL. In addition, the NRL participated in several other internationally organized PTs (Table 5.1).

Table 5.1 *Overview of proficiency and comparative tests, NRL metals and nitrogenous compounds*

PT	Analytes	Matrix	z-scores
FAPAS 07325	As, Cd, Pb, Hg	Animal feed	Between -0.2 and 0
FAPAS 07327	As, Cd, Pb, Hg	Milk powder	Between -0.1 and 0.8
FAPAS 07329	As, Cd, Pb, Hg, iAs	Canned crab meat	Between -0.3 and 0.3
FAPAS 07332	Cd, Pb, Sn	Grapefruit purée	Between -0.3 and -0.2
FAPAS 07334	As, Cd, Pb, Hg, Al, Cu, Ni	Barley flour	Between -0.1 and 0.7
FAPAS 07335	Cd, I	Infant formula	0.3 and -0.1, resp.
FAPAS 07337	Cd, Pb	Honey	0.1 and 0.3, resp.
FAPAS 07339	As, Pb, Cu	Vegetable oil	Between -0.3 and 0.2
EURL-MN PT-2019-01	As, Cd, Pb, Hg, I, iAs	Seaweed meal	Between -0.65 and 0.74
EURL-MN PT-2019-02	Cd, Pb, nitrate (NO_3)	Vegetable based baby food	Between -0.02 and 0.26, resp.
EURL-MN PT-2019-03	Cd, Pb, Hg, Ni, Cu	Offal	Between -0.5 and 0.05
FAPAS 3093	Melamine, cyanuric acid	Animal feed	0.1 and 0.4, resp.
FAPAS 15140	Nitrate (NO_3)	Rocket (rucola) puree	1.7

All results of the reported concentrations in the PTs mentioned above were satisfactory (z-scores between -2 and +2). After expanding the scope of the EURL-NRL network with nitrogenous compounds in 2018, EURL-MN PT-2019-02 was the first proficiency test organized by the EURL including a nitrogenous compound (nitrate). The Dutch NRL was rewarded by the EURL with a certificate for scoring in the top-3 laboratories with the lowest average z-score for EURL-MN PT-2019-02.

5.2 Assistance to official laboratories

5.2.1 Quality control

Analyses for the competent authority (CA) in milk and milk products are carried oud by an 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. All results of the OL were satisfactory, z-scores were within the range of -2 and +2.

5.2.2 Advise

Advising the OL for milk and milk products is on ad hoc basis and depends on the outcome of the comparison test organized two times a year by the NRL. In 2019, the results were good and no specific advice was given based on the comparison test. The NRL responded to questions from the OL on the analysis of melamine and cyanuric acid in milk.

5.3 Scientific and technical support to the competent authority

The NRL provided information to the competent authority on detection limits of lead in baby food, related to EU plans to lower the MLs. In addition, arsenic speciation was discussed with the CA, related to possibilities to the separate determination of the inorganic species arsenite and arsenate.

5.4 Contacts with other NRL's

During the EURL workshops, relationships with other NRLs were maintained. Furthermore, the Dutch NRL participated in a collaborative trial organized 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 Processing Contaminants

Coordinator: Liz Leenders

6.1 Activities within the EURL-NRL network

6.1.1 Participation in EURL-NRL workshops

In 2019, the Dutch NRL participated in the Workshop of the EURL for Processing Contaminants (PC) hosted at the National Food Institute at the Technical University of Denmark (DTU) on October the 10th and 11th. Over 30 participants attended the event, representing the 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. The outcome of the proficiency tests on PAHs in food supplements, acrylamide in coffee and furans in coffee organised by the EURL-PC in 2019 were presented and discussed. The number of participants in the PTs were highest for PAHs and acrylamide. Only a small number of NRLs participated in the furan PT, and more work on method development and implementation by other NRLs is needed. Three NRLs representatives gave presentations on their current activities regarding acrylamide (surveys, analytical approaches, mitigation strategies). A representative from DG SANTE (dr. Frans Verstraete) presented the recent and expected future developments of the EU legislation in the area of competence of the EURL-PC. He discussed analytical performance criteria which are now adopted for 3-monochloropropanediol (3-MCPD) in hydrolysed vegetable protein and glycidylesters (GEs) in vegetable oils. A recommendation for monitoring of acrylamide and furans is expected. He also mentioned that vegetable powders for smoothies may contain elevated PAH levels resulting from the drying process. The EURL-PC discussed the future PTs and other activities within the network for 2020.

6.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to processing contaminants in food in 2019 to participate in. The EURL is discussing whether to install such focussed working groups in the future.

6.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL includes PAHs, 2- and 3-mcpd esters and glycidyl esters, acrylamide and furans. The Dutch NRL participated also in PTs on these contaminants. In 2019 the NRL participated in the PTs organized by EURL and FAPAS (see Table 6.1).

Table 6.1 *List of PTs*

Name of PT	Topic	z-score
EURL	PAHs in food supplements	z-score between 0.3 and 0.7
EURL	Acrylamide in coffee	z-score of -0.1
FAPAS 0681	PAHs in olive oil	z-score between -0.3 and 0.9
FAPAS 0683	PAHs in cocoa butter	z-score between 0.4 and 1.0
FAPAS 0684	PAHs in smoked fish product	z-score between 0.1 and 0.5
FAPAS 2657	2- and 3- MCPD esters and glycidyl esters in potato crisps	z-score between -0.2 and 0.1
FAPAS 2658	2- and 3-MCPD esters and glycidyl esters in vegetable oil	z-score between -0.1 and 0.9

All results of the reported concentrations in the PTs mentioned above were satisfactory (z-scores between -2 and 2). The NRL has not participated in the PT for furans, because the WFSR analytical method was not yet established. The method development is foreseen in Q1 2020.

6.2 Assistance to official laboratories

6.2.1 Quality control

On request of the Dutch CA for milk and milk products (COKZ), the NRL performs analysis for PAHs in milk samples twice a year. In 2019, RIKILT and the laboratory of the NVWA were merged and the new institute WFSR was established. The former laboratory of the NVWA was an OL. Both former parties participated in the PT's for PAHs organised by FAPAS, and the z-scores were evaluated by the NRL. Furthermore, quality assurance was established by confirmation of PAHs concentrations in several samples analysed by the former OL.

6.2.2 Advise

Since the merger of the laboratories of the OL and the NRL, advice on analytical measurements, quality and measurement strategies are given on a regular basis. The NRL analysed official control samples of milk for the CA COKZ, advice to the CA was given on ad hoc basis.

6.3 Scientific and technical support to the competent authority

There has been frequent support to the ministries of LNV and VWS), NVWA and RIVM with regards to processing contaminants in food, bilaterally and in the working group on pollutants. In 2019, WFSR organized a PT on PAHs in meat, of which the report will be published in 2020.

6.4 Contacts with other NRL's

During the EURL workshop in Copenhagen there was a good exchange of information on analytical methods and experience and best practices with other NRLs. The relationships with other NRLs were maintained.

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 meeting on marine biotoxins was held in October 2019 in Baiona, Spain. Due to the foreseen Brexit, the British NRL was absent, also the Spanish NRL was absent. Paolo Caricato from DG Health and Food Safety (DG SANTE) represented the EU Commission. During the meeting, updates from DG SANTE were given, proficiency tests were discussed and the NRLs and EURL presented their activities.

News from the Commission by Paolo Caricato from DG SANTE.

- Some member states are still using the Mouse Bioassay (MBA) for the analysis of Paralytoc Shellfish Poisons (PSP). While this is still allowed according to the current EU legislation, DG Environment wants to discontinue the use of the MBA because alternative (chemical) methods are available. In addition, there is increasing pressure from animal welfare organisations to stop using the MBA. This topic will be discussed in the commission with the competent authorities of the various member states in November 2019.
- According to Decision 2002/226/EC, the scallop species *Pecten maximus* and *P. jacobaeus* with domoic acid concentrations exceeding 20 mg/kg can be put on the market if the contaminated parts are removed and the domoic acid concentration in the edible parts stays under the regulatory maximum limit. Some member states want to extend this policy to other scallop species and toxins. For PSP this will not be allowed due to the high risks associated with these toxins. Possible extension to other toxins and scallop species will also be discussed in November 2019.
- In the legislation, the PSP reference method referred to is the original publication of the OMA2005.06 (HPLC-FLD, so called Lawrence method). However, during the years more toxin have been added to the method and the NRLs use the extended method. This can pose legal problems in case of disputes. It was discussed how this discrepancy should be resolved.
- An EFSA survey on norovirus in oysters has been published, EFSA expressed the need for criteria on this subject. Therefore, this topic will be discussed in November, the industry will also be invited.
- PTX-1 and PTX-2 deregulation is being prepared.

Discussion on EURL-proficiency tests (PTs). In 2019, the EURL organised proficiency tests (PTs) for Amnesic Shellfish Poisons (ASP), lipophilic toxins and PSPs (see 7.1.3. for results WFSR). No tetrodotoxin (TTX) proficiency test was organised. The PSP proficiency test was performed with the reference HPCL-FLD method. The EURL and NRLs discussed the correct identification of some PSPs in more complex samples. The EURL has also analysed the proficiency test samples with the screening version of the reference method, which slightly overestimated the results of the full reference method. The EURL therefore advised that if the screening version is used, samples with a PSP concentration exceeding 600 µg STX-equivalents/kg should be re-analysed using the full reference method. The EURL has organised a training session for the screening version of the PSP method in 2019.

Miscellaneous. There are no developments in the EU Commission regarding TTX. A few NRLs have tested samples for TTX, the French NRL has found a few positive samples (around LOQ) out of 200 analysed samples, also in Spain, only three out of over 1000 samples were positive (TTX concentrations below LOQ). No TTX was detected in Norwegian and 503 Irish samples. The maximum concentrations found in Italy were 501 µg/kg in mussel and 331 µg/kg in oyster, these samples were taken in a shallow, relatively warm (18-20 °C) estuary. From all the NRLs only the Belgian NRL

intends to bring their TTX method under accreditation. The Dutch NRL already has the method under accreditation for some years.

The draft version of the phytoplankton guide has been sent out for final review, the microbiology and marine biotoxins guides are still in preparation. A draft version of these documents is planned for July 2020.

Finally, the EURL stressed the need for good communication and cooperation between competent authorities and NRLs.

7.1.2 Participation in working groups

In 2019 an EURL advisory board meeting was organised in February 2019 in Lisbon, Portugal. The Dutch NRL is part of this EURL advisory board. During this meeting the layout of the marine biotoxin guideline was discussed and how this guideline should be in-line with the recent developed phytoplankton guideline and the EURL guideline for the classification of production areas (microbiology guideline). The EURL will be in charge drafting a first version of the marine biotoxin guideline which will be discussed first in a restricted working group followed by discussing in a broader electronic working group with both representatives of the NRLs as well as the competent authorities. No other technical working groups were organised during the course of 2019.

7.1.3 Participation in proficiency and comparative tests

The NRL participated in the EURL PTs on ASP, PSP and lipophilic marine toxins in shellfish as well as PTs on PSP and TTX in shellfish organised by QUASIMEME.

For ASP two PT samples were analysed by the EU reference method (HPLC-UV). One of the reported results gave a z-score above |2|, which is an unsatisfactory result. The result of the other sample gave a satisfactory result (z-score < |2|). The cause of the unsatisfactory result was investigated and identified. Appropriate measures were taken, after recalculation of the z-scores after correction of the error all z-scores for ASP were satisfactory.

For PSP toxins three EURL PT and three QUASIMEME PT samples were analysed with the EU reference HPLC-FLD method (OMA 2005.06). In the EURL PT for the individual toxins 5 out of the 14 reported values had an unsatisfactory result (z-score > |2|). In the QUASIMEME PT 5 out of the 9 reported values gave an unsatisfactory result. 90% of the errors can be related to the recovery correction. When recalculating the results without recovery correction there is only one single unsatisfactory result for one of the PSP, GTX6 (z-score -2.6). For the analysis of GTX6, an hydrolysis step is needed in the sample preparation. Due to this hydrolysis step the measurement uncertainty is increased compared to the other PSP toxins which do not require this hydrolysis step. Furthermore, there is no recovery data available for GTX6 due to lack of certified standard solutions. When a standard with good purity and concentration becomes available the recovery data can be obtained and a recovery correction can be applied.

Three EURL PT samples were analysed for lipophilic marine biotoxins by the EU reference LC-MS/MS method. Samples contained okadaic acid group toxins, yessotoxin group toxins and azaspiracid group toxins. In total 17 results were reported with all z-scores < |2|. The NRL performed satisfactory for the total toxicity content in all samples (z-scores between 0.0 till 1.6) and also for all the individual toxins good results were obtained (z-scores -1.6 to 1.6).

The NRL also participated in a PT for TTX organised by QUASIMEME. The deadline for submission of results will close in February 2020, therefore at time of writing no results are available yet.

7.2 Assistance to official laboratories

7.2.1 Quality control

Due to the merger of the laboratory of the NVWA and RIKILT on the 1st of June 2019, WFSR was from that date the only official laboratory in the Netherlands. In the past the OL (the laboratory of the NVWA) was supported by RIKILT after participation in the PTs of QUASIMEME. Due to the merger and the fact that these PTs were organised in the 2nd half of 2019 this support was no longer needed.

7.2.2 Advise

The NRL was requested and provided early 2019 technical advice for the measurement of yessotoxins in shellfish to the OL early 2019.

7.3 Scientific and technical support to the competent authority

In 2019, 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 during the annual NRL meeting the competent authority (NVWA) as well as RIVM, WMR and, prior to June 1st, the laboratory of the NVWA were updated on the developments in the field of marine toxins.

7.4 Contacts with other NRL's

As the number of issues with marine biotoxins in shellfish was limited over the course of 2019, only limited interaction occurred between the various NRLs. The NRL for marine toxins participated in a cyanotoxin (freshwater toxins) workshop organised by the Belgium NRL for marine toxins. And the UK NRL visited WFSR for both a discussion and demonstration on our cell based methods which can be used as an alternative for the MBA.

8 National Reference Laboratory 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 7th until the 9th of May organised by the EURL BVL. This workshop consisted of a theoretical part and a practical part. The regular program included the National Residue Control Plan (NCRP) evaluations, news from the Commission, a discussion on the results of organized proficiency testing, and developments and news in the field of residues for which BVL is responsible (nitroimidazoles, coccidiostats, nonsteroidal anti-inflammatory drugs (NSAID's), beta-agonists and anthelmintics). Furthermore the following topics were give attention to: EFSA data collection / data evaluation; Measurement uncertainty; Multi-screening methods with HRMS instruments; Salicylic acid in milk – results of survey. NRL's from Latvia, United Kingdom, Norway and Poland gave an overview of their activities.

Secondly, the NRL participated in the EURL-Workshop in Fougères, held on the 19th until the 21st of June. This workshop For the Control of Antimicrobial Residues in Food from Animal was organised by ANSES. This workshop also consisted of a theoretical part and a practical part. Topics discussed in this Workshop were among others:

Regulatory Issues like: News from the Commission, News on Substances groups related to NRCP's evaluations for 2019-2020;

Analytical issues like: Veterinary Medicinal Products Residue (VMPR) Reference Materials; Evaluation of results from the EURL PT Program 2018; Multi-residue screening HRMS methods (Multi-residue method for screening multi-class VMPR's in meat and Multi-residue method for screening antimicrobial VMPRs in meat); Multi-residue confirmatory LC-MSMS methods (Metacolor project (dyes residue control);

Miscellaneous issues like: Group A6 prohibited substances in milk; beta-lactam residues in milk; nitrofuran metabolites in meat.

Thirdly, the NRL participated the EURL-Workshop in Wageningen, held from the 12th until the 13th of November. This workshop consisted of only a theoretical part. Some topics of this workshop were: Update EURL activities including Evaluations Member States NRCP results; Research study of A3 and A4 compounds in meat; NRL open forum with topics like: - The Role of Foodsmarthphones in future on-site testing, - Kicking HRMS into gear of Risk-based food monitoring, - A substance multimatrix multiresidue method using HRMS, - Mobile MS in Food Safety control, - Blade spray ambient MS; Summary discussion on the revision of Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of results.

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

8.1.2 Participation in proficiency and comparative tests

The NRL has participated in several proficiency tests organized by the EURLs and other international proficiency testing organizations and has obtained the following scores (see Table 8.1):

Table 8.1 *List of PTs and z-scores*

Description	Organizing institute	Z-score	Assessment
Non-Steroidal Anti-Inflammatory Drugs (NSAID's) in milk	EURL BVL	Between -0.7 and 0.8 Concordance 100%	Sufficient
Dyes (malachite green) in Fish	FAPAS	Between 0 and 0.6	Sufficient
Antibiotics in Milk – Screening and confirmation	EURL-ANSES	Between -0.5 and 1.1	Sufficient
Pharmaceutically Active Drugs in milk	EURL-BVL	Concordance 88%	Sufficient
Veterinary Drugs at low level in feed	Ducares	Between -1.9 and -0.2	Sufficient
A3 and A4 compounds in bovine muscle	EURL-Wageningen	Between -0.49 and 0.92	Sufficient
Antibiotics, Anthelmentics, Coccidiostats in feed	EURL-Wageningen	Between -1.19 and 0.26	Sufficient
Macrolides in meat (beef)	Progetto	Between -0.5 and 2.3	Deviate result *)
Beta-lactams in meat (beef)	Progetto	Between -0.81 and -0.48	Sufficient
Veterinary Drugs at low level in feed	Ducares	Between -0.4 and 0.8 Concordance 100%	Sufficient
Antibiotics in meat (beef)	WFSR	Between -0.66 and 0.56 Concordance 0% and 100%	Sufficient
Beta-agonists in liver	FAPAS	Between -1.2 and -0.2	Sufficient
Avermectines and beta-lactams in milk (low level)	Progetto	Between 0.07 and 0.73 Concordance 100%	Sufficient
A6 residues in Turkey muscle	EURL-ANSES	Between 0 and 0.1 Concordance 50%	Sufficient
Coccidiostats in chicken feed	EURL-JRC	Z-score 0.4 Concordance 100%	Sufficient
Synthetic hormones/steroids in urine	FAPAS	Between -0.1 and 0.8	Sufficient
Aminoglycosides and tetracyclines in kidney	FAPAS	Between 0 and 1.8 Concordance 100%	Sufficient
Nitroimidazoles and Avermectins in fish	EURL-BVL	Between -0.88 and 26.9 Concordance 100%	Deviate result *)
Avermectins and anthelmentics in liver	FAPAS	Between 0 and 0.7 Concordance 100%	Sufficient
Chloramphenicol in shrimp	FAPAS	z-score 0.8	Sufficient
Non-Steroidal Anti-Inflammatory Drugs (NSAID's) in milk	FAPAS	Between 0.5 and 17.2	Deviate result *)

*) Corrective actions taken in response to the deviate results

Total z-scores	70	% of total
neg z-score	25	36%
pos z-score	45	64%
z-score <=2 en >=-2	67	96%
Z-score <-2	0	0%
Z-score > 2	3	4%

Macrolides in meat; for the quantification of spiramycine the numbers of standard addition was adjusted, the calculated result then gave a z-score of 1.77. No further action was needed.

Nitroimidazoles and avermectins in fish; the standard solution used had a ten times lower concentration than was mentioned in the Standard Operating Procedure (SOP). This was the cause of the divergent z-score. No further action was needed.

NSAID's in milk; the calculation file contained the wrong addition levels, when the correct values were used the z-score was -0.2. No further action was needed.

8.2 Assistance to official laboratories

8.2.1 Quality control

Pursuant to the NRL tasks, the OLs are supervised. The NRL assures the quality of 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 OLs in case of problems or not corresponding results. Due to the merger of the laboratory of the NVWA (one of the OLs) and RIKILT June 1st of 2019 the control program will be changed in a second-line control program.

The NRL task (supervise the analysis of the OLs) is only focused on the analyte/matrix combinations from Group A (prohibited) and group B (regulated), mentioned in Annex I of Directive 96/23/EC. The current control program includes 35 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 one of the OLs were held in 2019 to inform one another of developments, discuss analytical issues, and establish corrective actions. This meeting also includes discussing the NRCP.

8.2.2 Advice

The Q3 meeting (consisting of the NRL and two OLs) was held twice in 2019 on the 12th of March and the 17th of September.

In 2019 the NRL participated in the Expert Meetings of Veterinary Medicinal Products in Brussels, which were held on the 3rd of March, the 14th of October, the 3rd of September and on the 3rd of December.

In 2019 the NRL participated in three meetings for the National Plan Residue control workgroup, these meetings were held on the 8th of April, the 15th of July and the 28th of October.

In 2019 the NRL participated in a meeting between WFSR and one OL with the topic ' the implementation of antibiotic analysis in dairy product which are exported to Russia', which was held on the 14th of March.

In 2019 one OL asked the NRL several questions, advice and some background information about salicylic acid in eggs, the reason for this were some suspected screening results of salicylic acid in eggs.

In 2019 a colleague of the NRL has attended the training 'Better Training for Safer Food' to become a National Expert in the field of auditing Residues Control Plans. This training was organised by the European Commission.

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 May in Fribourg (Switzerland). In one and a half day a range of topics was addressed, including a presentation and discussion of the 2018 proficiency test and the development of performance over the years, mass spectroscopy for specific materials, examination of insects, the use of mastermix for PCR, and the GTH marker. WFSR was present with two experts on light microscopy and PCR.

- *Performance of the NRLs for AP*. An overview of the performance for light microscopy of the NRL network was presented for the years 2006 to 2018, with emphasis on the last five years (2015-2018). The range in good performance rates was wide with two (out of 27) NRLs performing faultless in 10 out of 13 years down to one NRL with only one faultless year. The average number of faultless years was 6, with a median of 5 years. NRLs were anonymized. A clear improvement of the NRLs was visible in the overview. Two NRLs were faultless for the last five years. The years 2011 and 2013 were years in which the lowest number of NRLs were faultless (four NRLs), the year 2016 had the lowest number of faultless NRLs (eleven) in the last five years. The sample sets that were used to test the performance of the NRLs in the microscopic analysis over the years differed in degrees of difficulty. The EURL mentioned that this parameter was integrated in the reports to the Commission. For the three years mentioned, 2011, 2013 and 2016, in the test material hydrolysed feather meal was used as the adulterant, in two occasions with fish feed as matrix. Hydrolysed feather meal is difficult to observe. Very low spike levels, presence of insects and of star fish in test materials were other factors that made it difficult for NRLs to conclude correctly. The use of compound feed as standard matrix of the samples decreased over the years, with only three in the two years 2017 and 2018. These numbers include one blank (not spiked sample) of compound feed, as was included in every set of samples for negative control.
- *Insect detection*. The method for the microscopic detection of insects is based on a sedimentation with a combination of petrol ether and tetrachloroethylene, with a resulting density of 1.26 g/ml. The intention is that all ingredients of a compound feed will collect in the sediment and only chitin particles and muscle fibres will remain floating. The interlaboratory validation study was based on eight adulterated samples and one blank as negative control. The spike levels were 0.015% (1 sample), 0.1% (4 samples), 0.25% (2 samples) and 0.5% (1 sample) using one out of three selected insect species. Fifteen NRLs participated in the study, of which seven data sets were excluded from the final evaluation for false positives in the negative control, application of the wrong protocol and/or procedural errors. The overall results showed a Sensitivity of 0.94 after examination of two slides and 0.97 after examining three slides per sample. With the application of an LOD the accuracy dropped to 0.17 for both ways of examination. Especially the larvae of the black soldier fly (*Hermetia illucens*) were difficult to detect. The sample with 0.25% of *H. illucens* resulted in 25% of false negatives. Several reflections on the study design and results need to be made. Exclusion of datasets for false positive results of the blanks means that the quality parameter Specificity is not evaluated, although this is a necessary element in quality assurance as the other parameters are, such as Sensitivity. The highest counts of particles were (obviously) found at the spike levels of 0.25% and 0.5%, and in one of the samples at a level of 0.1%. The other samples hardly show results exceeding the LOD. There are good reasons to avoid the use of an LOD, but these results show that the sensitivity is relatively low.
- *PCR Mastermix*. Problems arose in the performance of the PCR due to the absence of Uracil N-glycosylase (UNG) in the current versions of the mastermix. WFSR collaborated with the EURL for testing and evaluating a new version including UNG (DMML-D2-U600) compared to the previous

version without UNG present (DMML-D2-D600). The first results show a good performance of the new version of the mastermix. Robustness still has to be established.

- *MS*. Several operational protocols for Mass Spectroscopy on ruminant feed and other types of feed are established, including UHPLC TripleQ for muscle peptides and for blood/milk peptides, Immuno enrichment/Nano-LC QqOrbitrap and a HPLC Q-TOF for untargeted examination. It is difficulty to fix LOD for ruminant PAPs (MBMs) for these methods; the LOD depends on the feed material used and on the heterogeneity of the material. There is a need of various pure species PAPs to evaluate the method (e.g. ovine, bovine, caprine). Options to achieve this are production of home-made PAPs. The organisation of an interlaboratory study between expert labs, and a discussion of the results during an expert meeting is planned. Late November samples were distributed to the members of the expert group (Italy, Germany, Norway and Belgium). Gelatin is a component for specific attention, but in the view of being a hydrolysed protein in the strict sense a relationship with the efforts to detect and identify hydrolysed proteins exists.
- *GTH*. Glycerol triheptanoate (GTH) is the legal marker for materials belonging to Categories I and II with a spike level of 250 mg/kg fat. JRC-Geel developed a GC-MS method of detection based on PAP. The sensitivity of the detection method depends on the fat content of the by-products used, the share of these by-products in feed and the resulting total fat content in feed. An implementation study at EU level is planned, to be organized by the EURL. A major aim would be to document the applicability of the current method for feed and organic fertilizers. Samples of fat were distributed to 19 participants of the implementation study. Results were collected in September and discussed during an expert meeting in November 2019. A report will follow.
- *Miscellaneous.* Several issues were discussed in the open discussion. The need to perform a second analysis in the microscopic detection of animal proteins is disputed. The specifications for the demand to carry out a second examination relates to the general provisions of Annex II in Regulation (EC) no 152/2009. A procedure developed by WFSR includes a second analysis of two additional subsamples when the initial result exceeds an analytical threshold. This procedure is intended to apply to visual methods, which are subjected to samples to be analysed by visual inspection (i.e. without using a microscope). In the case of microscopic examination reduced samples may be used, without a further indication of the need to perform repetitions. This division among two types of visual examination (with or without a microscope) was included in the original version of Annex II in Regulation (EC) no 152/2009 and is expected to be continued. The choice to perform a second analysis for the detection of animal proteins can be based on specific requirements. In general, a confirmatory analysis when a first result exceeds a specified threshold is common practice in feed safety control. In the view of a zero tolerance and for the lack of true replicates, a second examination of any result above zero is preferable.

9.1.2 Participation in Working groups

Working groups om GTH and MS were active, but the Dutch NRL did not participate in these groups (not all NRLs were invited to participate, including the Dutch NRL). Working groups on microscopy and PCR are currently not active.

9.1.3 Participation in proficiency and comparative tests

The final report of the 2018 proficiency test was issued in March 2019. In this PT for microscopy and PCR 26 NRLs (and some other laboratories that participated) received seven samples, four indicated as being aquafeed and three as being feed for terrestrial animals. Based on the SOP for deciding on the order of methods to be applied, either microscopy and/or PCR, three samples had to be subjected exclusively to microscopy, one exclusively to PCR and three to both methods. Two samples appeared to have the same content of porcine PAP (fish feed with 0.05% porcine PAP). This level of porcine PAP was not detected microscopically in 10 out of the 52 analyses (2 x 26) performed by the NRLs. Seven out of 26 NRLs were not able to microscopically observe an addition of 1% star fish in starter feed for broilers. The EURL indicated that six NRLs were underperforming in microscopy. One of these NRLs showed an overall score of 0.917 (a score of 1 would indicate fully correct results), whereas four other NRLs were accepted as sufficiently performing with the same overall score. Six NRLs were considered underperforming for their PCR results. Three of these six NRLs did not perform the ruminant PCR test because of their negative result after microscopy for the 0.05% of porcine PAP in the fish feed, a fourth did not perform the PCR test for the low amount of particles (below LOD). In this way a false

negative in the microscopic evaluation has implications on both the results of the microscopic detection and on PCR. The Dutch NRL reported correct results in all cases.

The 2019 proficiency test consisted of nine samples. Only four of these samples showed the purpose on their labels. Since the SOP for deciding the order of methods, either microscopy and/or PCR, is based on specific label information, for five samples it was difficult to decide which method to use. This issue will be evaluated after the release of the report in 2020.

9.2 Assistance to official laboratories

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

9.3 Scientific and technical support to the competent authority

The Dutch NRL has provided support to the competent authority (NVWA: Netherlands Food and Consumer Product Safety Authority) and to the Ministry of Agriculture, Nature and Food Quality in the process of relaxation of the ban on the use of animal proteins in feed. The topics addressed included the revision of the microscopic method as included in Annex VI of Regulation (EC) no 152/2009, the monitoring of poultry material needed in support of the anti-cannibalism ban and the monitoring of insects. Several notes were written by the Dutch NRL with background documentation on the mentioned topics.

WFSR is working on a set of monitoring methods for hydrolysed proteins, in cooperation with the NRLs of France and Poland. The competent authorities and the EURL were informed on the progress in this research by the Dutch NRL, this research will be continued in future.

The relationship between legal requirements and available analytical methods for monitoring animal proteins in feed was investigated. Good and workable definitions of animals included in legislation and insight in biological background of animal species appeared to be pivotal to this relationship. The results of the investigation including several recommendations were published in a scientific journal.

Support to the competent authority was provided whenever appropriate.

9.4 Contacts with other NRLs

Like the EURL AP and most NRLs the Dutch NRL is a member of the IAG (International Association for feedingstuff analysis) section for Feed Microscopy. One employee of WFSR is a board member of this section and also serves as scientific officer. During the annual meeting of IAG in June and if necessary during other meetings exchange of viewpoints and information is stimulated. WFSR 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.

Reference

L.W.D. van Raamsdonk, T.W. Prins, N. Meijer, I. Scholtens, M. Bremer, J. de Jong, 2019. Bridging legal requirements and analytical methods of animal proteins in feed for eradication of prion diseases. Food Additives and Contaminants, Part A, 36(1): 46-73. https://www.tandfonline.com/doi/abs/10.1080/19440049.2018.1543956

10 National Reference Laboratory Feed Additives and national evaluation of dossiers / advice

Coordinator: Jacob de Jong

10.1 Activities within the EURL–NRL network

10.1.1 Workshop

Wageningen Food Safety Research (WFSR) is the NRL both for 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). WFSR participated in both workshops, organized by the EURL, JRC-Geel (Belgium).

Feed Additives Authorization. The annual workshop for Feed Additives Authorization was organized from 22-23 October 2019 in Geel (Belgium). The workshop started with a training session for the evaluation of dossiers of feed additives. The aim of the training was to find the key information for four selected case scenarios (amino acids, trace elements' chelates, enzymes and mycotoxin binders) in order to complete an ad-hoc check list.

A representative from the European Commission (DG SANTE) gave an update about the status of the authorization of vitamin B2 as a follow-up to the detection of a GMO *Bacillus subtilis* production strain in a vitamin B2 additive.

A representative from the EURL presented the PCR methodology used at the JRC to verify the presence of recombinant DNA in vitamin B2 official control samples. The strengths and limitations of PCR methods were discussed.

Feed Additives Control. The annual workshop for Feed Additives Control was organized from 23-24 October 2019 in Geel (Belgium).

The results of the proficiency tests for carotenoids (colorants) and coccidiostats in compound feed were reported by the EURL. See below for the results of the Dutch NRL.

A representative from the European Commission (DG SANTE) gave an update about specific issues such as the on-going revision of Regulation (EC) no 152/2009 and about current developments regarding legislation for feed additives and undesirable substances.

The EURL informed the NRL-network that the development of an LC-MS/MS method for p-phenetidine in ethoxyquin and in fish meal is ongoing.

The NRLs were also updated on the outcome of the tests and developments performed for the determination of urea both as an additive in ruminant feed and as undesirable substance in non-ruminant feed and encouraged to participate into the related intercomparisons. The Dutch NRL participated in the PT for urea, applying an LC-MS/MS method.

The NRLs were informed that in 2020 a PT will be organised on the determination of vitamin D3 in feed.

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

In 2019 the NRL commented on 33 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 feed additives: technological additives (binders / anticaking agents, substances for reduction of the contamination of feed by mycotoxins), coccidiostats, sensory additives (flavoring compounds, colorants), zootechnical additives (gut flora stabilizers and other zootechnical additives) and nutritional additives (amino acids, trace elements, vitamins).

10.1.3 Participation in proficiency tests

The EURL for Feed Additives Control has organised proficiency tests (PT) for colorants, coccidiostats and urea. The methods applied were LC-UV/DAD for the colorants and LC-MS/MS for the coccidiostats and urea. The results are reported in Table 10.1. For urea the results are not reported yet by the EURL.

For the colorants in fish feed, for 1 out of 3 analytes, viz. adonirobin, an unsatisfactory z-score of 3.0 was obtained, most probably caused by the use of an old reference standard. The new reference standard was ordered prior to the proficiency test, but was not delivered in time.

For the coccidiostats at authorized levels, the results of WFSR were satisfactory in terms of the detection of the presence of the coccidiostats (diclazuril, narasin and nicarbazin) in the 2 samples; however, no quantitative values could be reported due to in-house problems with the quantification of coccidiostats at authorized levels. Work is on-going to solve these problems.

The NRL participated in a PT for coccidiostats, carbadox and olaquindox organised by Ducares, the Netherlands. WFSR applied an LC-MS/MS method. Monensin, nicarbazin, robenidin and salinomycin were detected and quantified with sufficient z-scores between -2 and 2 (see Table 10.1). Narasin, amprolium, carbadox and olaquindox were identified correctly. For these compounds the number of participants was too low to perform a statistical analysis.

The NRL also participated in a PT for nicarbazin, salinomycine and carbadox, organised by WFSR. An LC-MS/MS method was applied. Satisfactory z-scores were obtained, see Table 10.1.

Table 10.1 *Overview of results of PTs, NRL for feed additives*

PT	Analytes	Matrix	Z-scores
EURL	Colorants: astaxanthin, canthaxanthin, adonirobin	Fish feed	-0.7 – 3.0
EURL	Colorants: canthaxanthin	Poultry feed	-1.3
EURL	Coccidiostats at authorized levels	Poultry feed	-
EURL	Coccidiostats at cross-contamination level	Poultry feed	0.4
Ducares	Coccidiostats, carbadox and olaquindox	Pig feed	-0.4 – 0.8
WFSR	Coccidiostats and carbadox	Feed	-1.19 – 0.26

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 2019 22 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 coccidiostats, probiotics, enzymes, nutritional and zootechnical additives.

For 24 national requests it was evaluated if it concerned GMOs or additives produced by GMOs. In most cases it was concluded that the applications indeed concerned additives produced by GMOs. In those cases it was evaluated if there were specific concerns related to the safety for humans and animals and if the applicant submitted enough information to assess these aspects. In a limited number of applications, supplementary information was requested. In 2019 no application was rejected due to GMO safety aspects.

10.2.2 Other scientific and technical assistance

Among others, on request of the Dutch Ministry of Agriculture, Nature and Food Quality the NRL contributed to a survey from the European Commission to the national competent authorities (NCAs) to support the evaluation of Regulation (EC) no 1831/2003.

The Netherlands Food and Consumer Product Safety Authority (NVWA) was advised about the availability of methods that allow (i) the determination of bentonite and formic acid in premixtures and compound feeds and (ii) to analyze if a specific bentonite product (for reduction of the contamination of feed by mycotoxins) that contained more than 70% smectite was used for the production of the premixture and compound feed.

11 National Reference Laboratory GM Food and Feed

Coordinator: Ingrid Scholtens

11.1 Activities within the EURL – NRL network

11.1.1 Participation in EURL-NRL workshops

The Dutch NRL participated in the Steering Committee meetings of the European Network of GMO Laboratories (ENGL) in February and June 2019 (Ispra, Italy). At these meetings the 15th NRL and the 30th Plenary meeting were prepared (30th September - 2nd October 2019). The 15th NRL workshop consisted of three days: the NRL meeting, the ENGL plenary meeting and an open science day.

NRL meeting. At the NRL meeting an update was given on the comparative testing activities in 2019. Two proficiency tests (PT) were organised by the EURL in 2019. In the first PT tortilla crisps with NK603 maize and a Certified Reference Material maize 4114 were used as samples. The process of preparing the tortilla crisps was explained in detail. In the second PT of 2019 an animal feed containing three soybean GM events and one cotton GM event and a second sample consisting of soybean flour had to be analysed. Also a tour de table was held on NRL issues in 2019. Many laboratories were interested in a workshop on Next Generation Sequencing (NGS) and validation/accreditation. It was mentioned that digital PCR is taken up by more and more laboratories.

ENGL plenary meeting. At the ENGL Plenary meeting many topics were addressed e.g. the progress reports of the various ENGL Working Groups were given (see also 1.1.2). A new Working Group on GM microorganisms was announced and a report will be the final product of that Working Group. WFSR gave an update on the EUginius database, with emphasis on the ability to store information of gene-edited GMOs. Also a plenary discussion was held on Next Generation Sequencing and validation aspects of this technique.

Open science. The last day of the meeting was an open science day with interesting presentations from several countries. Some of the topics were: GMOs in India, metagenomics for plant and animal species, CRISPR-Cas systems, NGS overview of technologies, Probability of detection with the software package R.

Miscellaneous. WFSR also participated in an NRL Workshop on digital PCR quantification and conversion factors from copies/copies to mass/mass fraction, organized by the EURL GMFF in Geel, Belgium on 12th and 13th November 2019. The programme included 2 days of presentations, interaction and discussion. An introduction on digital PCR was given and the dPCR guidance document of the ENGL WG-dPCR was presented. Many of the current qPCR methods can be used in dPCR without significant changes. Some laboratories already have an accreditation for digital PCR methods and they explained their procedure to acquire the accreditation. Different approaches to acquire accreditation were discussed e.g. accreditation per GM event or accreditation by a description of the difference between qPCR and dPCR methods. dPCR is also used in many other applications like allergen detection, food authenticity testing, species quantification, microbiology, virology. The second day was dedicated to the proper use of conversion factors. A list of conversion factors has been published on the EURL website https://gmo-crl.jrc.ec.europa.eu/. Practical examples and exercises on the use of conversion factors in dPCR were performed in small groups.

11.1.2 Participation in Working Groups

Three web meetings of the multiplex PCR Working Group were attended in 2019 to further work on the draft document. A physical meeting is foreseen in February 2020 to finalize the document.

In November a physical meeting of the Working Group on DNA extraction was attended in Ispra. This Working Group will continue its work in 2020.

Also the ENGL Kick-off web meeting of the Working Group Method Performance Requirements was attended on 28th November 2019. The Working Group will review the current ENGL document 'Definition of minimum performance requirements for analytical methods of GMO testing' (MPR) and assess its applicability to digital PCR, new mutagenesis techniques (e.g. CRISPR/Cas9 genome editing) and food and feed derived from GM animals. The Working Group will reconvene in the beginning of 2020.

The ENGL Working Group on Sequencing has organized two teleconferences with the chapter coordinators, in April and October 2019. Based on the input of all members of the WG, a first complete draft report has been circulated to all members for comments. On 29-30 January 2020, a plenary meeting is scheduled to discuss the revised draft report, and to set the time path to finalization of the report.

11.1.3 Participation in proficiency and comparative tests

Two EURL proficiency tests were organised in 2019. In the first PT tortilla chips with NK603 maize and a Certified Reference Material of maize 4114 event were used as samples. In the second PT of 2019 an animal feed containing three soybean GM events and one cotton GM event, and a second sample consisting of GM soybean flour had to be analysed (see also 1.1.1). For WSFR it was important to have cotton included as a matrix, because it was a requirement of the Dutch board of accreditation, RvA, to also participate in a proficiency test for the matrix cotton. WFSR participated in the PTs with good results (Table 11.1).

Table 11.1 *Overview of PT results GMO detection in 2019*

Proficiency test	Analyte	Matrix	Z-score
ILC-EURL-GMFF-CT-01/19	NK603 maize	maize tortilla crisps	-0.7
ILC-EURL-GMFF-CT-01/19	4114 maize	maize flour	0.1
ILC-EURL-GMFF-CT-02/19	40-3-2 soybean	animal feed	-0.9
ILC-EURL-GMFF-CT-02/19	MON87708 soybean	animal feed	0.6
ILC-EURL-GMFF-CT-02/19	GHB119 cotton	animal feed	-0.2
ILC-EURL-GMFF-CT-02/19	DAS44406 soybean	soybean flour	0.8

11.2 Assistance to official laboratories

In June 2019 RIKILT merged with the laboratory of the NVWA (Lab VV) into the new institute WFSR. Before the merger the feed analyses were performed by RIKILT and the food analyses by Lab VV. So starting 1st June 2019 all GMO analyses, for food and feed, are performed by one institute, WFSR. Until June 2019 both laboratories (RIKILT and Lab VV) had their own accreditations. Therefore former-Lab VV also analysed the PT samples mentioned in 11.1.3 and the results were compared with former-RIKILT results and discussed. In the near future WFSR will work with only one accreditation for GMO analyses.

In spring 2019 assistance was also given to former-Lab VV for confirmation of GM rice analyses (SYBR Green method) and additional sequencing. This method is used for analyses of rice food from China (Implementing Decision 2011/884/EU, emergency measures regarding unauthorized GM rice in rice products originating from China, latest amended version).

11.3 Scientific and technical support to the competent authority

In 2019 a risk-based sampling strategy was used for the Dutch GMO feed monitoring program. The GMonitor module, developed by WFSR in 2015 (at that time still RIKILT) was used to determine the samples for the GMO analyses in feed. 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 GMOs that are not authorized to enter the EU.

11.4 Contacts with other NRL's

Contact with other NRLs in Europe took place during the Steering Committee meetings, the NRL meeting, ENGL Plenary Meetings in Ispra, Italy and the digital PCR workshop in Geel, Belgium. Since 2017 WFSR is also the NRL GM food and feed for Ireland. The NRL activities for Ireland are financed by Ireland in a separate project.

12 National Reference Laboratory 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 (MMP) was delisted from the list of EURL in Regulation (EC) no 882/2004. Without a formal EURL, no EURL-NRL workshops were organised in 2019. 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 financial support) from the Czech NRL MMP, the Dutch NRL joined a voluntary cooperative network of NRLs MMP, to replace parts of the former EURL activities. As a result from last years' enquiry of needs, the Czech NRL was able to organize the first international workshop of NRLs MMP, held in the Rapotin, Czech Republic, 27-28 March 2019, at which 13 representatives from 11 NRLs were present. In addition to a few general discussion on the working area of the NRLs and short presentations of each NRL on their further tasks and research in the field, there was room for two visits to specialised dairy producers in the vicinity. The NRLs agreed to cooperate and assist each other where possible, and an electronic sharepoint for NRL issues has been set up. Annual or biannual meetings are foreseen for the next years, for which the Czech NRL will take the lead.

12.1.2 Participation in proficiency and comparative tests

No EURL-PTs were organised in 2019, 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): ALP (Switzerland). January, May, September, 4 samples per round. Results are not known yet (February 2020).
- PT on somatic cell count (raw milk): Cecalait (France). March, June, September, December, 10 samples per round. Result z-scores mostly around -5.
- PT on somatic alkaline phosphatase (stabilised milk): LGC (UK). January, May, November, 2 samples per round. Result: z-scores between -0.3 and 0.9
- PT on total flora (raw milk): Cecalait (France). January, April, June, September, 10 samples per round. Result: z-scores between 0.4 and 2.5

One of the PT-providers for somatic cell count (Cecalait) calculates z-scores by taking combining results of laboratories using routine and reference methods. According to the Dutch NRL (and former EURL and other current NRLs), this makes the z-scores of this PT unrealistic. This PT is therefore only used to compare results of the NRL and those of the OL. The trueness of the somatic cell count generated by the NRL can be monitored from the Swiss-organised PT, for which the 2019 results are not yet reported, but have been good for all previous years. For total flora the agreement is that only z-scores <-3 or >3 are insufficient.

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2019, the NRL provided assistance to the Official Laboratory (OL) by providing reference results on the reference material for somatic cell count prepared by the OL, which is used to calibrate the routine equipment at the OL. This year, 8 series with 6 samples raw milk each, were analysed independently by two different technicians using the reference method. Twice, the NRL made a series of cheeses with a number of ALP levels for comparative testing between NRL and OL. For total flora it was arranged that the 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.

12.2.2 Advisory tasks

In 2019 the NRL had 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 mandated 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. The list of official analysis used by the mandated competent authority was received by the NRL mid-December 2019, and only a screening of available methods and their accreditation statuses by the NRL was possible.

13 National Reference Laboratory Moisture in Poultry Meat

Coordinator: Erika Silletti

13.1 Activities within the EURL-NRL network

The Dutch NRL on water content in poultry meat has taken part in two EURL-NRL expert meetings in 2019 together with the other European NRLs and the responsible representatives from Brussels.

13.1.1 Participation in EURL-NRL workshops

First meeting in Brussels
The first expert meeting took place on March 18th 2019 in Brussels. This meeting is the main meeting next to the meeting in the fall held in one of the member states. The meeting was opened by Alexander Bernreuther. The agenda was approved. Two topics were added under AOB: 'Preliminary German results on sample homogenisation with liquid nitrogen' (German NRL) and 'Ice-glazing of frozen chicken meat, wooden breast disease & water content in fresh chicken breasts' (Danish NRL).

Presentation Annual report to the CMO Committee in December 2018.
Alexander Bernreuther (DG JRC) showed his presentation previously given at the CMO (Common Market Organisation) Committee – sector animal products in December 2018. This presentation was based on JRC Technical Report entitled '2018 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 2018 in Dublin, Ireland.
Carlos Álvarez from the Department of Food Quality and Sensory Science of the Teagasc Food Research Centre (Ashtown, Dublin, Ireland) presented an overview of the activities at the Special Expert Group Meeting 'Monitoring of Water content in Poultry meat' held on 17-18 October 2018 in Dublin, Ireland.

Overview on control data by NRLs 2017.
In accordance with Articles 16, 18 and 20 of 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 2017 was presented by Alexander Bernreuther (DG JRC). Detailed statistical analyses and discussion on observations will be available in the '*2018 Annual Report of the Board of Experts in Monitoring Water Content in Poultry meat following Regulation (EC) No 543/2008*'. The numbers of reported data sets for poultry carcases and poultry cuts were similar to those of the previous years and a continuation of the trend from the last years towards improved consistency and completeness of reported data sets was reported. Concerning the over-the-limit matter, also in 2017 a large number of over-over-the limit cases were reported, in line with the previously observed trends.

Results of the 2018 proficiency test and potential follow-up of the 2016 study on the impact of sample homogenisation on the water content in poultry meat (Erika Silletti, WFSR).
Erika Silletti (WFSR, NL) presented the results of the evaluation of the 2018 proficiency test. The preliminary results presented in the meeting in Dublin in October 2018 were largely confirmed. Briefly, when considering both chicken fillets and drumsticks, analysed for both protein content and moisture content, only few labs did show satisfactory results. Concerning the set-up of a follow up of the 2016 homogenisation study, the first results of the inventory phase based on information already available at WFSR as provided by the NRL in 2016, were presented. The influence of the different temperatures used in the homogenisation procedure by different NRLs on the z-scores was illustrated. No clear trend

relating the temperature at the end of the homogenisation process with results observed in homogenisation study 2016 was found. Further information to be provided by the different NRLs is required.

Update on survey on national control systems in relation to the checks of water content in poultry meat under Regulation (EC) no 543/2008
Martin Szentivany (European Commission) presented the summary of the survey on national control systems of water content in poultry meat. This survey, undertaken last year, gathered basic information on the organisation of controls systems across Member States. 22 Member States contributed. The summary, in the form of Excel file, was shared with all NRLs in January 2019.
Martin Szentivany also informed the group on the state of play of the external evaluation of agricultural marketing standards. The evaluation was launched in December 2018 and its results can be expected at the end 2019/early 2020. It covers a broad range of agricultural marketing standards, including those for poultry meat (Regulation (EC) no 543/2008). An evaluation theme directly relevant for the Expert Group is the potential for simplification of controls systems, where the system of checks of water content will also be examined.

Information on the study on water protein ratios by Latvian poultry processors
Alexander Bernreuther (DG JRC) informed the participants about the official enquiry from the Latvian authorities regarding a recent study on water protein ratios. The main conclusions are that the average water protein ratios of Latvian chicken increased over the last years, and that a 4 hour fasting period prior to slaughtering of chicken seemed to significantly increase water protein ratios.

AOB
Gisela Hahn (German NRL) presented preliminary German results on sample homogenisation with liquid nitrogen on chicken carcasses. In an internal study, the routine/in-house method was tested in comparison to the procedure without liquid nitrogen or to a meat mincer. The use of liquid nitrogen (and consequently a low temperature of homogenates) in this study resulted in a slightly but significant higher protein content and in a lower W/P ratio. The data of the trial still needed to be completed and revised before being provided to the experts. Eric De Clerck (Danish NRL) introduced three current issues of the Danish NRL, i.e. Ice-glazing of frozen chicken meat in Denmark, Wooden breast disease (WBD) and Water content in fresh chicken breasts. The Danish NRL shared its concern with other MS and is in favour of a revision of the current marketing standards, i.e. an increase of certain legal limits for water/protein ratios of poultry meat. Commenting on this issue, Kai-Uwe Sprenger (DG AGRI) reminded the experts that control data should reflect the reality and that the current legislation has to be respected (R543/2008). He also reminded that currently the horizontal evaluation of agricultural marketing standards is ongoing and thus any revision of the poultry meat marketing standards cannot be expected before this process is finalised.
Alexander Bernreuther presented an enquiry from the Polish NRL, who found a new type of chicken cut on the market, i.e. skin and boneless chicken leg meat. The Polish NRL was asking for advice for an appropriate application of the legal W/P limits. The feedback form the experts was clear, underpinning that such a type of meat is currently not defined in Regulation (EC) no 543/2008 and consequently, there are no defined legal W/P limits for this particular cut.

Second meeting in Ljubljana, Slovenia
The fall meeting of experts was held in Slovenia on 7th and 8th November 2019 and was hosted by *National Veterinary Institute, Ljubljana, Slovenia*. Two excursions were planned on 7th November, i.e. the visit of a processing plant and of a broiler rearing farm of Pivka perutninarstvo. The excursion started with the visit of Pivka Poultry in Kal, the second largest poultry slaughterhouse and processing plant in the Republic of Slovenia, which belongs to the Delamaris group. The management of Pivka Poultry kindly provided insight in the organisation and operations of Pivka Poultry. The presentation of Pivka Poultry was complemented by an extended visit of the cutting and slaughter facilities. A broiler rearing farm was visited after the visit of the slaughterhouse. Discussions with supervisors at the production plant, a local veterinary inspector as well as with one of the company veterinarians provided a good level of understanding of the whole production chain.
The Expert group meeting was held on the 8th November. The first presentation was given by Zlatka Bajc, (NRL for water content in poultry meat, National Veterinary Institute, Ljubljana,

Slovenia). Zlatka Bajc, provided an extensive introduction of the institute which, with its 350 employees, acts as national reference laboratory (NRL) in nine domains in the area of food and feed safety. Amongst others, it hosts the NRL for water content in poultry meat, which assesses annually 30 samples of both fresh and frozen poultry parts for compliance with EU legislative limits on water content in poultry meat. The second presentation was given by Brigita Golob representing the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection (AFSVSPP). The AFSVSPP was established in 2013 and covers the whole agri-food chain. It provides food inspection and food control services for the Republic of Slovenia. Food inspection is executed by 10 regional offices and two boarder inspection posts. About 15 000 t of poultry meat are produced in the Republic of Slovenia in three major establishments, which are supplied by about 330 local breeders. The number of annually produced broilers remains rather constant at around 38 Mio, whereas the domestic turkey production is growing significantly. About one-third of the official control samples exceeded in the last three years the maximum levels for water content in poultry meat. These presentations were followed by two presentations given by Thomas Wenzl (European Commission) about the national control data from 2018, the first one, and the organisation of controls of water content in poultry meat under Regulation (EC) no 543/2008 in selected Member States, the second one.

Evaluation and interpretation of national control data from 2018 by Thomas Wenzl (European Commission)

Thomas Wenzel presented the first assessment of the 2018 control data. A detailed statistical analysis and discussion on observations will be available in the '*2019 Annual Report of the Board of Experts in Monitoring Water Content in Poultry meat following Regulation (EC) No 543/2008*'. The electronic version of this report will be provided to all participants. From the results, presented by Thomas Wenzel, it is worth to mention the following findings:

- The numbers of control data reported into the ISAMM database is rather stable at around 1000 for cuts and about 280 for carcasses. It has to be noted that some countries did not report any data for 2018, whereas other important poultry producing countries reported only few data into the database.
- Only seven out of 271 carcasses (2.6%) exceeded the threshold levels for water content, whereas 22 percent of poultry cuts were rated non-compliant. The vast majority of them comprised chicken breast fillets without skin imported from Brazil or Thailand.
- The distribution of water-protein ratios of samples produced within EU cannot be fully explained based on the available data and possibly required more data analysis and/or data acquisition.

Organisation of controls of water content in poultry meat under Regulation (EC) no 543/2008 in selected Member States (Thomas Wenzl, European Commission)

A pilot survey was conducted among four EU-Member States (EU-MS) in order to get insight into the organisation of controls of the water content in poultry meat within the respective EU-MS. The survey was addressed to public control laboratories (CLs)/competent authorities (CAs) responsible for the controls of the water content in poultry meat. The NRLs of the respective countries were used as vehicle for reaching the right persons. The survey comprised questions on the poultry production within the geographical area of responsibility of the respective CL/CA, as well as questions on communication and action taken in case of non-compliant samples. It finally gathered information on the sampling of poultry samples for the control of water contents in poultry meat. The survey was not considered as an audit and does not lead to consequences for the participating CLs/CAs. Some shortcomings in interpreting the questionnaire were identified. These will be resolved by improving the phrasing of questions, respectively by providing additional guidance. An update questionnaire will be distributed in the coming weeks to the NRLs for distribution to the concerned CLs/CAs (ACTION 2). The NRLs were requested to provide language support, if necessary.

Update on homogenisation project organised by Dutch NRL (WSFR) with the involvement of selected NRLs (Erika Silletti, WFSR, the Netherlands)

WSFR organised a study, in which the influence of sample homogenisation on water-protein content (W/P) ratios was tested. For that purpose, frozen chicken drumstick samples were sent to five NRLs for homogenisation. The homogenate had to be resent to WSFR for the determination of protein content and moisture. The procedures applied by the NRLs resulted in homogenates of different

textures. Homogenisation procedures involving liquid nitrogen for sample cooling resulted in lower water content and higher protein content values, and consequently lower W/P ratios compared to procedures omitting liquid nitrogen. The statistical analysis of the obtained data is ongoing. However, a first statistical analysis indicated that there were significant differences in water- and protein content when comparing labs which used liquid nitrogen with labs which did not have the use of liquid nitrogen in their procedure. The final report will be presented at one of the next meetings.

Update on future proficiency test organised by Dutch NRL (Erika Silletti, WFSR, the Netherlands)
In 2019, the NRLs did not express sufficient interest in participating in a proficiency test (PT) on the determination of the water content in poultry meat, which lead to the cancellation of the PT for this year. However, the organisation of a future PT potentially involving official control laboratories was discussed with the NRLs. The added value of such exercise would be to get a good level of understanding of the capabilities of control laboratories in the field, as ISAMM data showed that arbitration analysis did not confirm the results obtained at first instance in the majority of cases. Priority regarding participation in the PT will be given to NRLs. All other available places will be filled in the sequence of application. NRLs are requested to investigate whether official control laboratories in their countries would be interested in participating in 2020 in such PT. The PT will likely comprise chicken breast fillets.

EU poultry market in 2019: recent trends and outlook (Martin Szentivany, European Commission)
Martin Szentivany presented the development of the meat market in general and the poultry market in particular. The EU poultry production is still growing, despite reduction of production in some EU-MS. Prices of poultry meat produced within the EU were stable over the last years. However, they are still significantly higher than poultry meat produced in the US or in Brazil. Imports into EU are at the level of tariff rate quotas. Generally, the EU has a positive trade balance in poultry exports. During the discussions, participants mentioned that meat prices are likely to increase in the near future due to the massive outbreak of African swine fever in China, leading to losses of its domestic pork production and increased demand on the global market. The increased demand of meat is expected to affect also the poultry sector.

AOB
On request of the German NRL, a short discussion took place on the preparation of samples for chemical tests, which consist of whole carcases including giblets packed in plastic sachets in their abdominal cave. Consensus was reached that the plastic sachet needs to be removed before homogenization.

13.1.2 Organisation and participation in proficiency and comparative tests

No proficiency test was organised by WFSR in 2019.

13.1.3 Quality control

WFSR has organised two quality control rounds (March 2019 and August 2019) in order to ensure that the official laboratory and WFSR 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.

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.

13.2 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, 6,500 employees (5,500 fte) and 12,500 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.