National Reference Laboratories Wageningen Food Safety Research

Annual report 2020

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

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Wageningen, September 2021

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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 2020.

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 thirteen subjects. The tasks of a NRL depend on its research fields. This report gives an overview of the activities performed by all of NRLs of WFSR in 2020. These NRLs are for: milk and milk products, marine biotoxins, animal proteins, certain substances and residues thereof as laid down in Directive 96/23/EC, additives for use in animal nutrition (feed additives), genetically modified organisms (GMOs) in food and feed, pesticides, metals and nitrogenous substances in feed and food, mycotoxins and plant toxins in food and feed, processing contaminants, halogenated persistent organic pollutants in food and feed, food borne viruses 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 2020, due to COVID-19, most of these workshops were online. All workshops have been attended by NRLs of WFSR. 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 Union 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 obliged 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 has been officially designated as the NRL for 13 subjects by the Ministry of Agriculture, Nature and Food Quality (LNV) and by the Ministry of Health, Welfare and Sport (Medical Care) (VWS).

These subjects are (ordered as the EURLs in Annex VII of Regulation (EC) No 882/2004):
- Milk and milk products*
- Monitoring of marine biotoxins
- Animal proteins in feedingstuffs
- Residues of veterinary medicines and contaminants in food of animal origin (96/23/EC)
- Additives for use in animal nutrition and national evaluation dossiers
- Genetically modified organisms (GMOs)
- Residues of pesticides
- Metals and nitrogenous compound in feed and food
- Mycotoxins and plant toxins in feed and food
- Processing contaminants
- Halogenated persistent organic pollutants (POPs) in feed and food
- Foodborne viruses
- Moisture in poultry meat**

*: For 'Milk and milk products' the 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 NRL for moisture in poultry meat is appointed under the Single Market Regulation.

The objective of this report is to give an overview of activities performed by the NRLs of WFSR in 2020.

1.1 EU Legislation

The most important legislation in the EU on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules is the Official Control Regulation (OCR), Regulation (EU) 2017/625. 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 the Annexes of Directive 96/23/EC. For the official controls on the water content of poultry, additional provisions have been laid down in Regulation (EC) No 543/2008.

1.1.1 Competent authorities

According to the OCR Member States are to designate competent authorities responsible for official controls. In The Netherlands the ministry of LNV is the designated CA, this ministry mandated the Food and Consumer Product Safety Authority (NVWA) to perform some of the tasks of a CA. The NVWA is responsible for designating the official laboratories (OLs) for the analysis of official samples, samples taken for official control purposes. In addition, the NVWA 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 (EC). 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*

Subject/substances/products	EURL
Milk and milk products	*No longer required in the EU as of January 1, 2018*
Monitoring of marine biotoxins	Agencia Española de Seguridad Alimentaria (AESA) Vigo Spain
Animal proteins in feedingstuffs	Centre wallon de recherches agronomiques (CRA-W) Gembloux Belgium
Residues of veterinary medicines and contaminants in food of animal origin:	
For the residues listed in Annex I, Group A (1), (2), (3) and (4), Group B (2)(d) and Group B (3)(d) to Directive 96/23/EC *(A1 Stilbenes, stilbene derivatives, and their salts and esters, A2 Antithyroid agents, A3 Steroids, A4 Resorcylic acid lactones including zeranol, B2d Sedatives, B3d Mycotoxins in animal products)*	*Wageningen Food Safety Research* *Wageningen* *The Netherlands*
For the residues listed in Annex I, Group B (1) and B (3)(e) to Directive 96/23/EC and carbadox and olaquindox *(B1 Antibacterial substances, including sulphonamides, quinolones, B3e Dyes)*	*ANSES – Laboratoire de Fougères* *France*
For the residues listed in Annex I, Group A (5) and Group B (2)(a), (b), (e) to Directive 96/23/EC *(A5 Beta-agonists, B2a Anthelmintics, B2b Anticoccidials, including nitroimidazoles, B2e Non-steroidal anti-inflammatory drugs (NSAIDs))*	*Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)* *Berlin* *Germany*
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

Subject/substances/products	EURL
Residues of pesticides:	
Cereals and feedingstuffs	*Fødevareinstituttet* *Danmarks Tekniske Universitet* *København* *Denmark*
Food of animal origin and commodities with high fat content	*Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg* *Freiburg* *Germany*
Fruits and vegetables, including commodities with high water and high acid content	*Laboratorio Agrario de la Generalitat Valenciana (LAGV)* *Burjassot-Valencia* *Spain* *Grupo de Residuos de Plaguicidas de la Universidad de Almería (PRRG)* *Almería* *Spain*
Single residue methods	*Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart* *Fellbach* *Germany*
Metals and nitrogenous compounds in feed and food	National Food Institute, Technical University of Denmark Copenhagen Denmark
Mycotoxins and plant toxins in feed and food	Wageningen Food safety Research Wageningen The Netherlands
Processing contaminants	National Food Institute, Technical University of Denmark Copenhagen Denmark
Halogenated persistent organic pollutants (POPs) in feed and food	Chemisches und Veterinäruntersuchungsamt (CVUA) Freiburg Freiburg Germany
Foodborne viruses	Livsmedelsverket Uppsala Sweden
Water content poultry meat	Board of Experts: JRC (IRMM), DG AGRI and three NRLs*

* Note: 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 as mentioned in Table 1.1 are responsible for (article 94 (2) of Regulation (EU) 2017/625) the following tasks insofar they are included in their work programmes:

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 in feed, milk and poultry meat. A working plan describing the tasks for 2020 has been drafted in 2019. In addition, budgets for personnel, and facility and equipment costs have been drawn up. The working plans for 2020 have been positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of Agriculture, Nature and Food Quality (LNV) and by the Ministry of Health, Welfare and Sport (Medical Care) (VWS)) and has been approved by LNV. Working plans are based on NRL tasks as described in Regulation (EU) 2017/625.

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).

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

In some cases the NRLs are mentioned in EU legislation. RIKILT (as was the name of WFSR till June 2019) 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 ‘official laboratories’ (OLs) authorised to perform analyses of samples taken within the context of official controls. 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 Regulation (EU) 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/12682/2019	• Residues of plant protection products (all matrices)
Regulation (EC) 401/2006	• Mycotoxins in food
Regulation (EC) 333/2007	• Lead, cadmium, mercury, inorganic tin, inorganic As, 3-MCPD, 3-MCPD fatty acid esters, 3-MCPD glycidyl fatty acid esters, PAHs and acrylamide in food
Regulation (EU) 2019/627	• Marine biotoxins (paralytic and amnesic shellfish poison, lipophilic poisons, emerging marine biotoxins) • Some milk parameters (alkaline phosphatase activity, plate and somatic cell count)
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 milk and milk products

Coordinator: Martin Alewijn

2.1 Activities within the EURL-NRL network

2.1.1 Participation in EURL-NRL workshops

As of January 1 2018, the EURL milk and milk products (MMP) was delisted from the list of EURLs in Annex VII of Regulation (EC) No 882/2004. Without a formal EURL, no EURL-NRL workshops were organised in 2020. However, several member states, including the Netherlands, have expressed the intention to keep their NRL MMP active. NRLs of these states 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. After a first meeting in 2019, a second (online) meeting of NRLs was organised by the Czech NRL on 22 October 2020. This second meeting was attended by approximately 20 participants from 10 different countries. The workshop focussed on the way each of the NRLs performs their control tasks towards the official laboratories in their member states. Also some exchange between NRLs took place on their current dairy research to find opportunities for bi/multilateral cooperation. A draft document was made with concrete NRL MMP-related topics the involved NRLs would like to work on. This draft was phrased as a questionnaire and circulated by the Dutch NRL after the workshop, to focus the cooperation from 2021 onwards.

2.1.2 Participation in proficiency and comparative tests

As no EURL-PTs were organised in 2020, 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. Result: z-score between 0.6 and 1.3 (only the January result available at time of writing).
- PT on somatic cell count (raw milk): Cecalait (France). March, June, September, December, 10 samples per round. Result z-scores between -0.9 and 0.3 (WFSR was unable to complete the March-PT due to Corona-quarantine of staff at the time).
- PT on somatic alkaline phosphatase (stabilised milk): LGC (UK). January, May, November, 2 samples per round. Result: z-scores between -1.0 and 0.5.
- PT on total flora (raw milk): Cecalait (France). January, April, June, September, 10 samples per round. Result: z-scores between -0.6 and 1.9.

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. However, this PT organiser – and many of the other participants – now employ the JRC somatic cell reference material in their procedure, and in drastic contrast to previous years WFSR's results are now perfectly acceptable without any change in procedure at WFSR. WFSR has also analysed the JRC reference material, and found the results (repeatedly) to be within the assigned values, suggesting that analysis by WFSR sufficiently reflects the true somatic cell count.

2.2 Assistance to official laboratories

2.2.1 Quality control

In 2020, 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, 7 series with 6 raw milk samples each, were analysed independently by two different technicians using the reference method. The NRL re-analysed a series of routine cheese samples from the OL cheeses to compare the alkaline phosphatase levels obtained by 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.

2.2.2 Advice

The NRL advised the competent authority and the official laboratory on the process of implementing the reference material on somatic cells (JRC ERM-BD001) into routine analyses. In 2020 the NRL had meetings with the NVWA on the need for official control on official routine analyses in dairy products. With the new Control Regulation (EU) 2017/625 in force since December 2019, the current scope of NRL quality control on the dairy routine laboratories needed to be reviewed, and possibly expanded. The competent authority (COKZ) has a wide range of analyses performed at its official laboratories, and the NRL assisted in categorising the different analyses and issues to be resolved. As part of this new work, the NRL applied for accreditation for a few extra products. Discussions on the mode for NRL-quality control of the new tasks were undertaken, but some decisions on supporting the competent authority in fulfilling their supervisory task towards the official laboratories remain to be decided in 2021.

3 National Reference Laboratory Marine Biotoxins

Coordinator: Arjen Gerssen

3.1 Activities within the EURL-NRL network

3.1.1 Participation in EURL-NRL workshops

The NRL participated in the EURL workshop, held online on November 18th and 19th 2020. A representative from DG Health and Food Safety (DG SANTE) of the EU Commission was present at the meeting. During the meeting, updates from DG SANTE were given, proficiency tests were discussed and the NRLs and EURL presented their activities.

News from DG SANTE.

- Pectenotoxins will soon be deregulated, revision of the legislation is accepted.
- The Commission wants to enhance the traceability of live bivalve molluscs that are placed on the market. To achieve this, the Commission wants a common approach to placing shellfish on the market, including common formats of registration documents which are electronically available.
- The Commission stated that they prefer the CEN method for Paralytic Shellfish Poisoning (PSP) toxins as official reference method instead of the EURL standard operating procedure (SOP). This sparked an intense debate, as most of the NRLs preferred to use the EURL SOP. A working group was formed from the NRLs, which prepared the opinion of the NRLs on this point. This opinion should be used as input for the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF) meeting on December 10th 2020. The Dutch NRL suggested to move towards performance criteria-based methods instead of standardised methods, as is common for methods in other food safety areas.
- The trading agreement between the US and EU is formalised (EU-US equivalence). It is expected that the first trade in live bivalve molluscs will be between the US and Spain and US and The Netherlands.

Discussion on EURL-proficiency tests (PTs). In 2020, the EURL organised proficiency tests (PTs) for Amnesic Shellfish Poisoning (ASP), lipophilic toxins and PSPs (see 3.1 for results WFSR). For ASP the overall performance was very good. For lipophilic toxins, most results were satisfactory. Only in one of the two samples, one participating NRL had an unsatisfactory score for total okadaic acid (OA). For PSP toxins, there were some issues with the correct identification of some individual toxins. When the laboratories which incorrectly identified some toxins were excluded, the results for total toxicity were satisfactory in most cases.

Miscellaneous. WFSR presented some of the work on marine and cyanobacterial toxins, such as the detection of tetrodotoxin (TTX), Diarrheic Shellfish Poisoning (DSP) toxins and cyanobacterial toxins in shellfish from official production sites. TTX was detected above the Dutch regulatory limit (44 µg/kg) in both the Oosterschelde and Grevelingenmeer. This was the first time that concentrations above the regulatory limit for TTX were detected outside the Oosterschelde. In Veerse Meer, potentially DSP toxin producing phytoplankton was detected during one month, and in this period, DSP toxins were detected in shellfish. The highest detected concentration was 130 µg OA-eq/kg, which is below the EU regulatory limit of 160 µg OA-eq/kg. The cyanobacterial toxins microcystins (MCs), anatoxins and cylindrospermopsins (CYNs) are not regulated in shellfish and are not routinely monitored. In a survey with samples from 2019, however, traces of anatoxin and MCs were found in a few samples. CYNs were detected in 60 of the 165 analysed shellfish samples from different production areas. The highest detected CYN concentration (sum CYN and 7-deoxy-CYN) was 64 µg/kg.

The NRL of France reported the presence of anatoxin-a in seafigs, in concentrations up to 1200 µg/kg. Analysis was performed as a reaction to a food poisoning incident. In Spain, yessotoxins have been detected in the scallops (Mimachlamys varia). High PSP concentrations in shellfish were reported by Norway (7000 µg/kg) and Ireland (2500 µg/kg). The Irish NRL fears that the PSP problems are getting more widespread in Ireland. High levels of ASP were reported by Ireland (580 mg/kg in inedible parts of scallops), Norway reported a maximum concentration of 20 mg/kg. In Greece, the DSP toxin OA was above the regulatory limit in 30% of the tested samples. Germany and Belgium validated their method for TTX, the Belgian method is also accredited. The Belgian NRL tested algal supplements for the presence of the cyanobacterial toxins microcystins. These toxins were found in 8 of the 35 analysed samples, the highest concentration was above 1 µg/g. The French NRL is screening shellfish for nonregulated toxins, including cyanobacterial toxins.

The EURL gave updates on the progress of the phytoplankton guide, which is finished. The biotoxins monitoring guide is in progress, two of the expected six chapters are completed. The microbiological control guide is in its initial stage.

3.1.2 Participation in working group

In 2020, an EURL advisory board meeting was organised in February in Madrid, Spain. The Dutch NRL is part of this EURL advisory board. During the meeting the progress of the marine biotoxins monitoring guide and progress on the EURL SOP for PSP toxins was discussed. The marine biotoxin guide is drafted by the EURL with support of the advisory board. Currently, two of the six chapters are ready and are open for revision by the NRLs. These chapters contain the sampling plans and a description of monitoring of unclassified areas. The draft chapters about the sampling protocol, analytical methods and interpretation of data are foreseen for 2021.
Furthermore, in this meeting in Madrid, the extension of the EURL reference method towards a rapid screening method for PSP toxins was discussed. This method is already used as EU reference method for confirmatory analysis. With some small adjustments it could also be used as a more rapid screening method. The advisory board discussed the content and revised the first version of the extended SOP. Subsequently, the SOP was open for revision by all the NRLs. The further implementation of the SOP is currently on-hold as the Commission took the decision to remove the EURL SOP from the legislation and decided to move forward with CEN procedures in the legislation for marine biotoxins.

3.1.3 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 with the EU reference method (HPLC-UV). One of the reported results gave a z-score above |2|, which is unsatisfactory. The result of the other sample was satisfactory (z-score < |2|). The cause of the unsatisfactory result was investigated and identified. In the initial result the sum of domoic acid (DA) and some potential epimers of DA (epi-DA) was reported. When solely reporting DA all z-scores for ASP were satisfactory. The procedure is adjusted to avoid misreporting in the future.

For PSP toxins, two EURL PT and six QUASIMEME PT samples were analysed with the EU reference HPLC-FLD method (OMA 2005.06). In the EURL PT for the individual toxins, 1 out of the 9 reported values had an unsatisfactory result (z-score > |2|, Table 3.1). In the QUASIMEME PT 18 out of the 24 reported values gave an unsatisfactory result (Table 3.2). All unsatisfactory results can be related to the recovery correction that WFSR applies. Still most of the laboratories do not apply a true recovery correction within their analysis. The Dutch NRL is of the opinion that a recovery correction should be applied and therefore submitted the results as would have been reported in official analyses. However, to evaluate the performance of the Dutch NRL in comparison to the other laboratories the Dutch NRL also calculated the results without recovery correction. Without application of the recovery correction, all results would have been satisfactory (Tables 3.1 and 3.2).

Table 3.1 *z-scores of the EURL PSP toxin PT with- and without recovery correction*

Sample	Toxin	Assigned Value	σ	Ux	Unit	Initial results		Recalculated results	
						Value	z-score	Value	z-score
EURLMB/20/P/01	Total toxicity	1700	246	31	µgSTXdiHCl-eq/kg	2000	1.29	1370	-1.18
	GTX2&3	530	103	46	µgSTXdiHCl-eq/kg	690	1.47	484.6	-0.37
	STX	1100	171	38	µgSTXdiHCl-eq/kg	1300	1.16	885.8	-1.16
EURLMB/20/P/02	Total toxicity	1100	189	65	µgSTXdiHCl-eq/kg	1200	0.50	910	-1.11
	dcGTX2&3	65	22	16	µgSTXdiHCl-eq/kg	93	1.03	56.6	-0.30
	C1&2	250	61	35	µgSTXdiHCl-eq/kg	400	2.18	259.1	0.09
	dcSTX	150	34	15	µgSTXdiHCl-eq/kg	190	1.32	134.0	-0.28
	GTX5	200	41	11	µgSTXdiHCl-eq/kg	250	1.12	169.7	-0.81
	GTX6	310	74	43	µgSTXdiHCl-eq/kg	290	-0.27	291.1	-0.27

Table 3.2 *z-scores of the Quasimeme PSP toxin PT with and without recovery correction*

Sample	Toxin	Assigned Value	Total error	Unit	Initial results		Recalculated results	
					Value	z-sore	Value	z-score
QST284BT	dc-STX	0.55	0.122	µmol/kg	0.69	1.15	0.48	-0.58
	GTX-2,3	5.2	0.78	µmol/kg	7	2.36	4.87	-0.37
	STX	1.3	0.22	µmol/kg	1.6	1.23	1.08	-0.95
	Total toxicity	1800	240	µgSTXdiHCl-eq/kg	2400	2.38	1666	-0.68
QST285BT	GTX-2,3	6.3	0.89	µmol/kg	10	4.41	7.12	0.88
	STX	2.0	0.32	µmol/kg	2.7	1.96	1.83	-0.64
	Total toxicity	2200	288	µgSTXdiHCl-eq/kg	3300	3.57	2273	0.09
QST286BT	C-1,2	2.8	0.42	µmol/kg	5.4	6.26	3.45	1.50
	dc-GTX-2,3	0.36	0.141	µmol/kg	0.64	1.99	0.39	0.22
	dc-STX	0.34	0.097	µmol/kg	0.55	2.14	0.38	0.39
	GTX-2,3	0.89	0.177	µmol/kg	1.5	3.31	1.03	0.77
	GTX-5	4.1	0.58	µmol/kg	5.7	2.85	3.93	-0.25
	STX	0.38	0.1	µmol/kg	0.51	1.31	0.35	-0.28
	Total toxicity	700	97	µgSTXdiHCl-eq/kg	1400	6.74	836	1.35

Two EURL PT samples were analysed for lipophilic marine biotoxins by the EU reference LC-MS/MS method. Samples contained OA group toxins, yessotoxin group toxins and azaspiracid group toxins. Thirteen results were reported, which all had z-scores < |2|. The NRL performed satisfactory for the total toxicity content in all samples, as well as for all the individual toxins.

WFSR participated in a development exercise (DE) for TTX organised by QUASIMEME. Both submitted results were unsatisfactory (z-score > |2|). However, good results were obtained after re-analysis. The DE was performed with a less robust method. This method was routinely applied in 2019 and up to mid-2020. In 2020 after method development, the method was replaced by an improved more robust method. This method is used for another TTX DE, the results of this exercise will become available during 2021.

3.2 Assistance to official laboratories

WFSR is the only Dutch official laboratory for marine toxins. Therefore, no quality control had to be performed and no advice had to be given to other official laboratories.

3.3 Scientific and technical support to the competent authority

The NRL provided advice to the competent authority on deregulation of pectenotoxins and on the PSP reference method. The NRL supported the deregulation of pectenotoxins. For the PSP reference method, the NRL advised to keep the current EURL SOP as the official method. The reason for this advice is mainly the flexibility of the EURL SOP. This is very useful when for example new toxins are available or improvements to the method should be made. Unfortunately, the Commission decided to replace the EURL SOP by the CEN procedure. The NRL also provided the competent authority background information on a ciguatoxin intoxication. The NRL assisted the risk assessment department of NVWA (NVWA-BuRO) and RIVM in the risk assessment of the cyanobacterial toxins MCs and CYNs. In addition, in another project, WFSR is performing a study for NVWA-BuRO on the presence of cyanobacterial toxins in Dutch surface waters. The results of this study are due in March 2021.

During the annual NRL meeting the competent authority (NVWA) as well as VWS, RIVM and Wageningen Marine Research (WMR) were updated on the developments in the field of marine and cyanobacterial toxins.

3.4 Contacts with other NRL's

Together with the NRLs of France, Italy, Greece, Portugal and Ireland the Dutch NRL prepared an opinion on maintaining the EURL SOP for PSP toxins in the legislation as official method. Furthermore, the NRL received a request for brevetoxin occurrence data by the NRL of France. After approval of the competent authority data of a survey held in 2018 was shared with the NRL France.

4 National Reference Laboratory animal proteins

Coordinator: Leo van Raamsdonk

4.1 Activities within the EURL–NRL network

4.1.1 Participation in EURL-NRL workshops

The annual meeting of the EURL/NRL network was not organised due to the COVID-19 pandemic. A virtual meeting as alternative was not held.

4.1.2 Participation in Working groups

Working groups on Glycerol Triheptanoate (GTH, a substance used to permanently mark animal products unfit for entering the foodchain) and Mass Spectrometry (MS) were active, but the Dutch NRL was not invited to participate in both. Working groups on microscopy and Polymerase Chain Reaction (PCR) are currently not active.

4.1.3 Participation in proficiency and comparative tests

The 2019 proficiency test (PT) organised by the EURL consisted of nine samples. Only for four of these samples the purpose of use was shown on their labels. For the other five samples it was difficult to decide on the correct method for examination since the Standard Operational Procedure (SOP) for deciding the order of methods, (either microscopy and/or PCR), is based on specific label information. The report on this PT was released in 2020 and showed that the microscopic and PCR results of the Dutch NRL were correct in all cases. However, errors had been made by the EURL in the process of evaluating the results of all NRLs. These errors were partly related to the contents of the mentioned SOP. It was not possible to discuss this in the absence of the annual meeting. After a discussion with the staff of the EURL, which did not result in correction of the draft nor of the final version, six NRLs filed a complaint. The initial final version of the report was withdrawn and replaced by a final version.

The 2020 proficiency test organised by the EURL consisted of eight samples. Two samples were present as duplicate. Any selection of appropriate methods, either microscopy or PCR, was not requested and consequently all samples were examined by both methods. The Dutch NRL reported correct results when using microscopy for all samples and seven correct and one not correct results for samples that were analysed using PCR. The four signals making up a final result were randomly distributed around the detection limit for the non-correctly reported sample. Such a single situation does not require immediate action. The overall microscopic result of all NRLs for a sample of aquafeed containing, according to the EURL, 0.1% of pig material was 0.538 (a score of 1 would indicate all results to be correct), which is an indication of false negative reports of 12 out of 26 NRLs. This particular aquafeed with pig material had been used in three earlier PTs with at least moderate results (over 80% correct). The EURL will start a survey to find out the possible reasons for the deviant microscopic results for this aquafeed in this PT.

4.2 Assistance to official laboratories

For animal proteins in feed there is only one OL in the Netherlands (WFSR). Therefore, no official activities are performed for this task.

4.3 Scientific and technical support to the competent authority

Support to the competent authority was provided whenever appropriate.

The EURL started the finalising of the microscopic method for inclusion in Annex VI of Regulation (EC) No 152/2009. There were several issues in this new version of the method, which were not solved. A main issue is the full fixation of the embedding agent for making the microscopic slides. In contrast to chemical methods, where the detection unit is part of the analysing equipment, the microscopic technician is carrying out the detection visually, based on his or her experience. The embedding agent used will influence the microscopic appearance of the animal particles, and should therefore match the technician's experience. Therefore some NRLs argued that the embedding agent used should be free to choose, however the EURL decided to continue to prescribe two specific agents. After confirmation in the technical working group and the Standing Committee, the amendment of Annex VI of Regulation (EC) No 152/2009 (Implementing Regulation (EU) 2020/1560) was published at the end of 2020.

Within weeks after the amendment was published, the EURL distributed a first draft of a combined method for Processed Animal Protein (PAP) detection and insect detection. Issues for the collaborative study for validation of the insect detection method were described in the NRL Annual Report of WFSR over the year 2019. A main issue is the obligation to use petroleum ether as one of the solvents. The safety sheet of this solvent includes the hazards 'highly flammable' and 'teratogenic effects'. An already tested alternative is heptane, which has a safety profile comparable to the commonly used solvent tetrachloroethylene. A discussion of this issue did until now not result in a relaxation of the EURL of the rules on the use of certain chemicals.

WFSR is working on monitoring methods for hydrolysed proteins. The Competent Authority has requested to develop a road map for the monitoring and enforcement of the large variety of animal by-products commercially available and listed in the relevant Regulations. Hydrolysed proteins, insects, gelatine and blood products are among the types of material which cannot be characterised by the only currently existing microscopic method: the PAP method. It is therefore necessary to develop and maintain a set or toolkit of dedicated methods for animal proteins, comparable to the already existing situation in the chemical domain.

WFSR is developing Quality Guidelines for visual inspections of feed and food in the view of absence of any Standard for quality assurance and quality control in the visual domain. This lack is one of the basic gaps causing problems as indicated in the previous paragraphs. WFSR, also as host of the Dutch NRL for animal proteins, has the intention to provide a broad basis for the quality of methods for visual inspection.

4.4 Contacts with other NRLs

WFSR serves as scientific officer and as board member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are member of this section. During the IAG annual meeting in June every year and if necessary during other meetings exchange of viewpoints and information is stimulated. WFSR on behalf of the board organises the annual IAG proficiency test (PT) 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. The IAG PT for animal proteins was not organised due to the COVID-19 pandemic.

The cooperation with the NRLs of France and Poland for the development of monitoring methods for hydrolysed proteins in the framework of a research project at WFSR was continued.

5 National Reference Laboratory 96/23/EC

Coordinator: Johan Lasaroms

5.1 Activities within the EURL-NRL network

5.1.1 Participation in EURL-NRL workshops

Due to the COVID-19 pandemic the EURLs had decided to postpone the annual workshops to the end of 2020 so that these workshops could be held 'live on location', but eventually it was decided by the EURLs that the workshops would be online meetings.

The NRL participated in the EURL-Workshop organised by ANSES-Fougeres, which was held online on the 13th of October and the 14th of October and which was also the first online annual Workshop. The workshop for the 'Control of Antimicrobial Residues in Food from Animal Origin' consisted only of several presentations.

Some topics of this Workshop were: 'Regulatory Issues like: News from the Commission, News on the EURL Website Services; EURL evaluation of the National Residue Monitoring Plans (NRMP) 2020 (List of recommended substances for Group B1 (antibacterial substances, including sulphonamides, quinolones) and Group B3e (dyes) (Annex 1 Directive 96/23/EC) and an introduction to the evaluation of EU-MS RMP 2020; Evaluation of the results from the EURL-PT Program 2019.

The topic of one of the PT's was the control of banned substances (chloramphenicol, nitrofurans and nitroimidazoles residues) in turkey muscle. The aim of the PT was to evaluate of the participants could detect, confirm and quantify these residues using their routine method. Some specific issues about the compound group nitrofurans were explained (like the washing step for the bound residues) and also that too few participants (13 out of 28) were able to detect the compound DNSH (3,5-dinitrosalicylic acid hydrazide, the side-chain of the nitrofuran nifursol).

In the field of high resolution mass spectrometry (HMRS) a presentation was given with the topic 'advances in HRMS methods for Veterinary Medicinal Products Residue in Meat and Milk'. The strategy including the advantages and disadvantages of using HRMS for residue analysis of antibiotics for official control was explained.

A presentation was given about the 'advances in tracking antibiotic residues in feathers'. The context and objective of this study was to investigate the possible routing of residues from a treated animal to other species, animals or the environment. The focus was on the distribution or the occurrence of residues in feathers and feather meal and via these matrices the distribution to other animals or the environment.

Secondly, the NRL participated the EURL-Workshop organised by WFSR-Wageningen, which was held online on the 3rd of November. This workshop for the control of Growth promoters as Residues in Food from Animal Origin consisted of only a theoretical part.

The scope and aims of the workshop were:
- Bringing together NRLs and discussing topics in the field of growth promotors
- Discuss and give input for new legislation
- Get an insight in trends in the field
- Exchange knowledge between the NRLs
- Establish a long lasting collaboration between the NRLs

Some interesting topics of this workshop were:
'State of Play with regard to Thiouracil', which include the explanation of the background on the use of thiouracil and the outcome of an animal trial. This animal trial has yielded a lot of data with which could explain the thiouracil levels during and after treatment; thiouracil phase I and II metabolites, unique markers and a reliable strategy to discriminate between thiouracil abuse and endogenous thiouracil in feed materials.

'Broad steroid screening by GC-HRMS', whereby the focus was on the added value of GC-HRMS related to the possibilities of using a multimethod, the untargeted data acquisition, retrospective data analyses and risk based monitoring.

'Possible contamination at the laboratory', were an insight was given were the possible risk of a contamination could occur in a laboratory and if there was an overall cleaning approach possible.

Thirdly, the NRL participated in the EURL-Workshop organised by BVL-Berlin, which was held online on the 7th of December until the 9th of December. This workshop consisted of only a theoretical part including different presentations.

Some interesting topics of this workshop were:
'News on substance groups of the EURL', for the substance group anthelmintics new compounds or class of compounds which were included in the analytical method were listed, for the substance group nitroimidazoles an optimised and validated method for the matrix muscle was presented, for the beta-agonists the method development for the matrix lung was presented and an overview of the method development and validation of amitraz and its metabolites in egg was presented.

'Toltrazuril residues in Eggs – Result of an authorised application?', where the focus was on the pharmacokinetics of toltrazuril in laying hens. Some conclusions of this study were: Toltrazuril residues were detected, even though the withdrawal period was respected; high residues of toltrazuril were found in eggs, also after end of withdrawal period; statistical analysis indicates that it takes at least 56 days until 95% of the eggs contain residues below CCa.

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

5.1.2 Participation in proficiency and comparative tests

The Dutch NRL has participated in several proficiency tests organised by the EURLs and other international proficiency testing organizations and has obtained the following scores (Table 5.1):

Table 5.1 *Overview of results of proficiency tests, NRL 96/23/EC*

Description	Organising institute	Z-score	Assessment
Thyreostats in Bovine urine	EURL-Wageningen	Between -0.83 and 1.64	Sufficient
Coccidiostats in Egg	EURL-BVL	Between -0.87 and -0.04	Sufficient
Ureum in Animal Feed	EURL JRC	Between 1.0 and 2.0.0 Concordance 100%	Sufficient
Parasiticides and Antibiotics in Salmon	WFSR	Between -0.32 and 0.36	Sufficient
Glucocorticoids in Bovine Milk	FAPAS	Between -1.4 and -1.3	Sufficient
Chloramphenicol in Bovine Milk	Progetto	Z-score is 0.0	Sufficient
Nitrofurans in Porcine meat	Progetto	Between -0.51 and 0.27	Sufficient
Avermectines in Bovine Liver	Progetto	Z-score is -0.18	Sufficient
Macrolides in Porcine meat	Progetto	Between 0.53 and 0.71	Sufficient
Quinolones in Egg	Progetto	Between -1.02 and -0.24	Sufficient
Beta-agonists in Bovine hair	EURL-BVL	Between -0.2 and 0.6	Sufficient
Synthetic steroids in Bovine urine	Progetto	Between -0.42 and 3.5 Concordance 100%	Deviate result *)

Description	Organising institute	Z-score	Assessment
Avermectines and beta-lactams in bovine milk	Progetto	Between 0.5 and 1.32	Sufficient
Beta-agonists in porcine meat	FAPAS	Between -1.3 and -0.6 Concordance 100%	Sufficient
Dyes in Fish	FAPAS	Between -0.2 and 1.1	Sufficient
Avermectines and anthelmintics in Sheep liver	FAPAS	Z-score is 1.8 Concordance 100%	Sufficient
Tranquilizers in Porcine liver	FAPAS	Between -1.4 and -1.1	Sufficient
Phenicols in Bovine milk	FAPAS	Z-score is 0.1 Concordance 100%	Sufficient
Coccidiostats and chloramphenicol in chicken feed	FAPAS	Z-score is 1.1 Concordance 100%	Sufficient
Antibiotics, anthelmintics and coccidiostats in animal feed	WFSR	Between -1.26 and 5.02	Deviate result *)
Antibiotics in Porcine meat	WFSR	Between 0.23 and 0.42 Concordance 100% False Negative result	Deviate result *)
Chloramphenicol in shrimp	FAPAS	Z-score is 0.3	Sufficient

Cumulative results are presented in Table 5.2 below.

Table 5.2 *Cumulative results PTs*

Total z-scores	74	% of total
neg z-score	30	41%
pos z-score	44	59%
z-score <=2 en >=-2	71	96%
Z-score <-2	0	0%
Z-score > 2	3	4%

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

Synthetic steroids in Bovine urine; quantitative result of the compound 17alpha-boldenone differs from the assigned value.
Corrective action: the reported value was checked and the conclusion was that the reported value was not the calculated value of 17alpha-boldone. Using the correct value of 17-boldone the conclusion was that this was comparable with the assigned value. No further action was needed.

Antibiotics, anthelmintics and coccidiostats in animal feed; with the applied analytical method, which should be suitable for all the requested compounds, the quantitative results of lasalocid and narasin differs from the assigned value.
Corrective action: the proficiency sample will be reanalysed with the method specific for the detection and quantification of coccidiostats in animal feed, it is expected that the quantitative result for lasalocoid and narasin will be comparable with the assigned value. No further action was needed.

Antibiotics in Porcine meat; a false negative result of cefalexin was reported.
Corrective action: the raw data of the analysis was checked and it could be concluded that for the component cefalexin the wrong retention time was chosen. When the correct retention time was chosen the result was that cefalexin was present in the sample. No further action was needed.

5.2 Assistance to official laboratories

5.2.1 Quality control

Pursuant to the NRL tasks, the OLs are supervised. The NRL assures the analyses of the OLs 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.

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 1 of Directive 96/23/EC. The current control program includes 32 analyte/matrix combinations. A monthly evaluation of the outcome of this control program takes place and an annual trend analysis based on those results is performed and reported separately.

Four Technical meeting between the NRL and the OL were held in 2020 to inform each other of developments, discuss analytical issues and establish corrective actions. This meeting also includes discussing the control program.

5.2.2 Advice

The Q3 meeting (consisting of the NRL, OL and another laboratory) was held once in 2020 on the 30th of June. This was the 125th meeting between these institutes.

In 2020 the NRL participate in four meetings for the National Plan Residue control workgroup, these meetings were held on the 2nd of March, the 8th of June, the 21st of September and the 7nd of December.

In 2020 the NRL participated in a meeting between WFSR and another laboratory with the topic ' the implementation of antibiotic analysis in dairy product which are exported to Russia', which was held on the 13th of February.

In 2020 the OL asked the NRL to perform additional analyses for the confirmation of the identity of 17alpha-methyltestosterone, this because some samples had a deviate result on a possible finding of 17alpha-methyl-testosterone.

In the period from the 3rd until the 10th of March 2020 the Competent Authority (NVWA) was audited on the Residue Control Plan by the European Commission/DG SANTE. A representative of the Dutch NRL was involved in this audit to explain the role and the activities of the Dutch NRL related to the OL and the CA of the Netherlands.

6 NRL Feed Additives and national evaluation of dossiers / advice

Coordinator: J. de Jong

6.1 Activities within the EURL – NRL network

6.1.1 Participation in EURL-NRL workshops

Wageningen Food Safety Research (WFSR) is the NRL both for Feed Additives Authorisation (Regulation (EC) No 1831/2003) as well as for Feed Additives Control (Regulation (EU) 2017/625). The Dutch NRL participated in both workshops, organised by the EURL, JRC-Geel (Belgium). The annual workshops were organised as on-line meeting from 24-25 November 2020.

A representative from the Dutch NRL presented the problems that are encountered with the analysis of ionophore coccidiostats and nicarbazin at additive levels in feed. Applying the CEN-multi-analyte method, several experimental conditions were optimised. However, more work is needed in order to develop a method for the correct quantification. Several NRLs commented that they have similar problems. The EURL promised that follow-up discussions will take place on the analysis of coccidiostats.

A representative from the Austrian NRL reported about problems with the analysis of vitamin A in feed. Since 2018, an increasing number of results below the declared value was observed in the official controls of many Member States. An intensive root cause analysis was able to identify three potential main causes, viz. (i) changes in formulation of vitamin A due to the suspension of ethoxyquin; (ii) impact of feed production and feed composition; (iii) representative sampling and the influence of grinding.

A representative from the European Commission (DG SANTE) gave an update about the on-going revision of Commission Regulation (EC) No 152/2009.

A representative from the EURL reported about the progress of their project for the development and validation of methods for the determination of antibiotics at cross-contamination levels in feed. This is a contribution to the EC action to fight antimicrobial resistance, where maximum levels of cross-contamination for 24 antibiotics will be included in medicated feed legislation (Regulation (EU) 2019/4). The Dutch NRL contributes to this project by means of supplying information on methods of analysis that are used at WFSR.

The NRLs were also updated on the outcome of the proficiency test for the determination of urea as undesirable substance at low levels in pet food. The primary aim was to investigate which type(s) of methods are suitable. It could be concluded that only LC-MS/MS methods are fit-for-the purpose. This information has been integrated in the advice of CEN/TC 327 Animal Feedingstuffs to the European Commission regarding the method of analysis that should be standardised in a future Standardisation Request. The Dutch NRL participated in this proficiency test, applying an LC-MS/MS method; the results are described in par. 6.1.3.

A representative from EFSA reported about the tasks, activities and challenges of the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) that reviews the dossier information submitted by applicants and examines the efficacy and safety of the additive in terms of animal and human health as well as the environment.

6.1.2 Dossier evaluation on request of the EURL for Feed Additives Authorisation

In 2020 the Dutch NRL commented on 30 initial evaluation reports prepared by the rapporteur laboratory. The evaluation concerned the methods of analysis that were submitted in the dossiers. The advices were described in evaluation reports and included the following additives: technological additives (binders / anticaking agents, substances for reduction of the contamination of feed by mycotoxins, hygiene condition enhancers, substances for control of radionuclide contamination, silage additives, preservatives), coccidiostats, sensory additives (flavouring compounds, colourants and substances that add or restore colour in feedingstuffs), zootechnical additives (gut flora stabiliers digestibility enhancers, substances which favourably affect the environment and other zootechnical additives) and nutritional additives (amino acids, their salts and analogues, trace elements and vitamins).

6.1.3 Participation in proficiency tests

The EURL for Feed Additives Control has organised a proficiency test (PT) for urea. The method applied by the NRL was LC-MS/MS. The lowest concentration in the PT, viz. 18 mg/kg, was reported as below the limit of quantification. For the samples with concentrations of 249 and 935 mg/kg satisfactory results were obtained (see Table 6.1).

The NRL participated in a PT for coccidiostats at cross-contamination levels in feed, organised by FAPAS (UK). WFSR applied an LC-MS/MS method. Amprolium was detected and quantified with sufficient z-scores between -2 and 2 (see Table 6.1). For monensin and narasin, the results were satisfactory in terms of the detection of the presence of the coccidiostats; however, no quantitative values could be reported due to in-house problems with the quantification. Work is on-going to solve these problems.

The NRL also participated in a PT for antibiotics and coccidiostats at cross-contamination levels in feed, organised by WFSR. LC-MS/MS methods were applied. For blank samples, satisfactory results were reported, viz. no false-positive results were obtained. For all four antibiotics (oxytetracycline, chlortetracycline, florfenicol and tylosin), satisfactory results were obtained, see Table 6.1. For the two coccidiostats (lasalocid and narasin), unsatisfactory z-scores were obtained, see Table 6.1. Work is on-going to solve the quantification problems.

Table 6.1 *Overview of results of PTs, NRL for feed additives*

PT	Analytes	Matrix	Z-scores
EURL	Urea	Pet food	1.0 – 2.0
FAPAS	Coccidiostats at cross-contamination levels: amprolium	Poultry feed	1.1
WFSR	Antibiotics and coccidiostats at cross-contamination levels:	Feed	
	• Antibiotics		- 1.3 – 1.6
	• Coccidiostats		2.2 – 5.0

6.2 Scientific and technical assistance to the competent authority

6.2.1 Evaluation of applications for temporary use exemptions of non-authorised feed additives

In 2020 29 national requests for permission to use substances – which are not authorised 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, nutritional, technological, sensory and zootechnical additives.

For 25 national requests it was evaluated if it concerned GMOs or additives produced by GMOs. In a select amount of 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 2020 no application was rejected due to GMO safety aspects.

6.2.2 Other scientific and technical assistance

Among others, the Dutch delegation in the Standing Committee in the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF), Section Animal Nutrition, consisting of the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) and the Netherlands Food and Consumer Product Safety Authority (NVWA), was advised about the status of certain products as feed additives within the scope of Regulation (EC) No 1831/2003 and on the withdrawal from the market of certain feed additives.

The Dutch delegation in SCoPAFF was also advised whether attapulgite and chabazite should be regarded as feed additive or feed material.

WFSR participated in two meetings of the Dutch committee for authorisation of feed additives (APR), where among others advice was given about the requirements for flushing and the destination of flushing feeds in the framework of the evaluation of applications for temporary use exemptions of non-authorised feed additives.

7 National Reference Laboratory GM Feed and Food

Coordinators: Ingrid Scholtens (2020), Theo Prins (2021)

7.1 Activities within the EURL-NRL network

7.1.1 Participation in EURL-NRL workshops

The Dutch NRL participated in the Steering Committee meetings of the European Network of GMO Laboratories (ENGL) in February (Ispra, Italy) and June (web meeting). At these meetings the 16th NRL and the 31st ENGL Plenary meetings were prepared (29th September - 30th September 2020). The 16th NRL workshop consisted of a NRL meeting (one day) and an ENGL meeting (one day).

NRL meeting. At the NRL meeting an update was given on the comparative testing activities in 2020. Due to COVID-19 one instead of two proficiency tests (PT) were organised by the EURL in 2020. In this PT, bird feed fat balls containing MON88017 maize and a maize flour containing GA21 maize were used as samples. Also, a tour de table was held on NRL issues in 2020. Many laboratories were interested in a workshop on digital PCR. The Dutch NRL mentioned the importance of the developments in gene edited plants and animals, as well as the issues with detection of DNA of GM microorganisms in enzyme preparations.

ENGL plenary meeting. The ENGL plenary meeting was held on one day instead of the usual two days and was organised by Webex. The meeting was attended by three representatives of the Dutch NRL. It consisted mainly of interesting contributions of scientists on topics like gene-editing, GM animals and insects, and Genetically Modified Microorganisms (GMM).

Miscellaneous. WFSR participated in an NRL Workshop on calculation of measurement uncertainty, organised by the EURL GMFF by Webex on 12th and 19th November 2020. The programme included a thorough theoretical explanation on the scientific basis of the expanded measurement uncertainty, and a practical exercise, of which the results were discussed in the second meeting.

7.1.2 Participation in working groups

The Dutch NRL is chair of the Working Group (WG) DNA. The WG progress was at a slow pace in 2020 due to COVID-19 and no physical meeting was held. Several online meetings were organised.

Three web meetings of the multiplex PCR Working Group were attended in 2019 to further work on the draft document on multiplex PCR. A physical meeting was foreseen in February 2020 to finalise the document, but this meeting was not organised due to COVID-19. The ENGL Working Group on Multiplex PCR delivered a final draft document by the end of 2020. This document will be sent to the ENGL after approval by all members of the WG.

A physical meeting was also foreseen for the WG Minimum Performance Requirements Part 2 (MPR2) but instead, a web meeting was organised in March 2020. The WG MPR2 focused on the applicability of the guidance set out in the minimum performance requirements document and proposed amendments, when necessary, for digital PCR, detection of gene edited products, and detection of genetically modified animals. In 2020 a first draft of the 'Definition of minimum performance requirements Part 2' was written, a final version is scheduled to be finished in 2021. The Dutch NRL participates in this working group, as member of the subgroup focusing on the detection of genetically modified animals.

The ENGL Kick-off web meeting of the WG Genetically Modified Microorganisms was attended on 10th November 2020 by two representatives of the Dutch NRL. During the kick-off meeting the chair was elected for this WG and together with the attendees the mandate was discussed to establish the focus of this WG. Several points were raised by the attendees, such as the fact that ~50% of GMMs are fungi, and that no recombinant DNA is supposed to be present in products produced with or derived from GMMs. The WG prepared a first outline for the draft document, focusing on regulation regarding the detection of GMMs used in/for food and feed products. A second meeting of the WG GMM is planned for early 2021.

The Dutch NRL contributed to the virtual meeting of the ENGL Working Group on Sequencing on 25th November 2020. In 2020 a first draft was finished titled 'Sequencing strategies for the traceability of GMOs – methods and related quality aspects'. The final draft is scheduled to be finished in 2021.

7.1.3 Participation in proficiency and comparative tests

One EURL proficiency tests was organised in 2020. In this PT bird feed fat balls containing MON88017 maize, and a maize flour containing GA21 maize were used as samples. The challenge with the bird feed fat balls was that this material contains a lot of fat (see also 7.1). A second PT that was foreseen in 2020, has been postponed to 2021. The Dutch NRL participated in this PT with good results (Table 7.1).

Table 7.1 *Overview of PT results GMO detection in 2020*

Proficiency test	Analyte	Matrix	Z-score
GMFF-20/01	MON88017 maize	bird feed fat balls	-0.74
GMFF-20/01	GA21 maize	maize flour	-1.06

7.2 Assistance to official laboratories

In the Netherlands WFSR is the only official laboratory for GMO food and feed analyses.

7.3 Scientific and technical support to the competent authority

In 2020 a risk-based sampling strategy was used for the Dutch GMO feed monitoring programme. 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 authorised to enter the EU. This module is used by the competent authority in the monitoring of the presence of non-authorised GMOs in feed.

WFSR organised a Workshop on synthetic biology for the competent authority and ministries, and Mini Workshop on GMM. The Dutch NRL also advised the competent authority and ministry of LNV on issues regarding the difficulties that currently exist on the export of animal feed to the Russian Federation.

7.4 Contacts with other NRLs

Contact with other NRLs in the EU took place during the Steering Committee meetings, the NRL meeting, ENGL Plenary Meetings in Ispra, Italy and the NRL workshop on measurement uncertainty. 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. For the 4-year period of 2021-2024, WFSR will continue to be the NRL GM Food and Feed for Ireland.

8 National Reference Laboratory Pesticides in food and feed

Coordinator: Hans Mol

8.1 Activities within the EURL-NRL network

8.1.1 Participation in EURL-NRL workshops

For pesticides there are four EURLs, three covering a type of commodity (FV = fruit & vegetables; CF = cereals & feed; AO = products of animal origin & high fat content), and one covering pesticides that are not amenable to multi-residue analysis and need dedicated single residue methods (SRM). WFSR is NRL for pesticides in food and feed (all four EURL domains).

In the EURL-NRL network, one or more workshop are held every year. In odd years this is a joint event by all four pesticide EURLs, in even years separate meetings are held by the individual EURLs.

In 2020, two EURLs organised a separate workshop on their own, while the two others organised a joint workshop. Due to COVID-19 all workshops were held as online meetings, the programmes were shorter and the interaction and bilateral discussions and exchanges were obviously less lively in comparison to the physical meetings.

The purpose of the workshops 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, comments and input on the guidance document 'analytical quality control and method validation procedures for pesticide residue analysis in food and feed' were inventoried and discussed.

For each of the four workshops, the items presented and discussed are briefly summarised below:

EURL workshop FV [University Almeria, Spain; 29-30 October 2020, online]

- *Presentation of EURL-proficiency tests (PTs).* Three PTs had been organised in 2020. One on quantitative multi-residue analysis of 208 pesticides (plus an additional 36 voluntary, based on SANCO/12745/2013) in onion. The PT was open for NRLs and OLs, altogether 176 participated. In total 19 pesticides were present in the range 0.02 to 1.2 mg/kg. Most pesticides were measured and reported by the majority (80-97%) of the laboratories, but a few were only reported by 69%, 52%, or even only 12% of the laboratories. Quantification was satisfactory for more than 90% of the laboratories for the different individual pesticides. A notable exception was fenpicoxamid (only 62% of the laboratories with satisfactory results). The interlaboratory variability (RSD_R) varied from 15% to 23%, average 19%. The RSD_R appears to be constant over the past five years, indicating the measurement uncertainties are more or less constant now. The second PT concerned a 'special commodity' (a commodity considered to be more difficult for analysis), in this case avocado, to be analysed for 205 pesticides. In total 22 pesticides were present, which included four relevant metabolites. Levels ranged from 0.007 to 0.35 mg/kg. In total 62 laboratories participated. Also in this PT, not all laboratories covered all pesticides (e.g. spirotetramat-enol metabolite only 56% of the laboratories). Acceptable quantitative performance was obtained by 68%-100% of the laboratories. The RSD_R varied from 15% to 48% (average 22%), reflecting the higher complexity of the matrix. The third PT was rapid screening analysis (qualitative, quantitative optionally) of pesticides in onion. In this case, no target list was given, laboratories had to detect 'any' pesticide and report within 72 hours. In total 17 pesticides were present (all between 0.03-0.10 mg/kg), including several long-banned and some very recently authorised pesticides. Eleven laboratories out

of 62 detected all 17 pesticides, indicating that it is still difficult to find 'any' pesticide in a sample. Fluacrypyrim (relatively new, not authorised in EU) was only detected by 32% of the laboratories.

- *Three technical presentations by the EURL were given*. The EURL presented the use of supercritical chromatography coupled to tandem mass spectrometry (SFC-MS/MS) as an option to improve determination of captan and folpet. Details can be found in Cutillas et al. [Talanta (2021) 223, 121714]. The EURL shared its experiences with 'dual layer injection' in gas chromatography-based analysis. Essentially this was an in-syringe preparation of matrix-matched standards, improving sensitivity and peak shape, but also resulting in occasional extra interferences. Also a presentation was given on dual-channel chromatography where a double set up of the liquid chromatographic system was coupled to one mass spectrometer. It required a more sophisticated LC system and dedicated software control, but the mass spectrometer was then used more efficiently (less idle time) resulting in higher throughput (135 versus 80 injections in 24 hours).
- *The NRL Germany (BVL) reported on the 'German metabolite project'*, a joint project of the German NRL/OLs to include pesticide metabolites in multi-residue methods (MRM). Reasons to do that included: requirement in current residue definition, expected inclusion in future residue definition, and as alternative for complex residue definitions (e.g. common-moiety definitions like for iprodione). The availability of analytical reference standards was an issue but this situation is improving. In total 55 metabolites were assessed for inclusion. Implementation and actual use in routine sample analysis varied for the different OLs. Formal reporting was not always possible. Certain metabolites were not amenable to MRM methods, others were never found. The ones considered most relevant (MRM amenable and detected) included CGA 304075 (cyprodinil), prochloraz metabolites (BTS40348, BTS44959, BTS 44596), and Fluopyram-benzamide (fluopyram). *Note: WFSR also worked on this topic in 2020, partly looking for the same metabolites, but also additional ones including metabolites for which no standards are yet available. Several metabolites were detected.*
- QA/QC. In the EU, for official analysis of pesticide residue in food and feed a guidance document (SANTE/12682/2019) on validation and analytical quality control needs to be followed. Comments and input to the document were collected and discussed. This serves as input for the bi-annual update of the document foreseen for 2021.

EURL workshop CF/SRM [DTU, Copenhagen, Denmark/CVUA Stuttgart, Germany; 21-22 October 2020, online]

- This was a joint workshop from the EURL-CF and the EURL-SRM, since the PT matrix in 2020 was the same for both EURLs (rice).
- *Presentation of EURL-proficiency tests*. The PTs organised in 2020 dealt with the analysis of rice. The one organised by the EURL-CF was on quantitative multi-residue analysis of 164 pesticides (plus an additional 38 voluntary) in rice kernels. The PT was open for NRLs and OLs, in total 164 participated. Nineteen pesticides were present, partly incurred and partly spiked, in the range 0.01 to 0.40 mg/kg (mostly around 0.05 mg/kg). Most pesticides were measured and reported by the majority (80-92%) of the laboratories, but a few (oxathiapiprolin and endrin-ketone) were reported by less than 40% of the participants. Quantification was satisfactory for more than 90% of the laboratories for most pesticides. Exceptions included the two pesticide mentioned before. A number of the laboratories that did measure them had unacceptable results. Dichlorvos also appeared to be more troublesome. The interlaboratory variability (RSD_R) varied from 16% to 25%, average 19% (so similar as for fruit and vegetable matrices, see above). The PT organised by the EURL-SRM covered 13 mandatory and 17 voluntary pesticides, that are troublesome in MRM methods, or can only be analysed using a dedicated SRM method. In total 14 pesticides were present in the samples, including glyphosate, chlormequat, paraquat, several esters and a glucoside of phenoxyacid herbicides, and carbosulfan. In total 110 laboratories participated. For carbosulfan and the esters/conjugates of acidic herbicides high variability of results was observed which was attributed to the different hydrolysis conditions used by the laboratories. The interlaboratory variability (RSD_R) varied from 16% to 52%, average 25% which is higher than for the multi-residue methods, reflecting the higher degree of difficulty.
- *Four technical presentations were given*. The NRL-CF of Italy presented on the activities of the Italian NRL on SRM methods, a.o. the organisation of national PTs and analytical method development. The EURL-CF presented results on an automated micro-SPE cleanup coupled to GC-Orbitrap-MS for multi-residue analysis of pesticides in cereals. The cleanup proved to be efficient

and reduced labour. On the other hand limitations in the choice of micro-SPE columns and some practical issues were encountered. The EURL-SRM provided an update on method development, validation and application of SRM methods. Apart from the existing SRM pesticides and their technical issues, special attention paid to ethylene oxide. For this pesticide RASFF notifications were reported for sesame seeds originating from India since late August 2020. Ethylene oxide is a fumigant that can be used to control insects and microorganisms in dry food products (herbs, spices, nuts, oil seeds). It can also be used for sterilisation of medical equipment and disinfection of storage facilities, wood, wools and furs. In the EU, the use of ethylene oxide for the disinfection of foodstuffs, e.g. in storage areas, is not permitted. The MRL has been set to 0.05 mg/kg in sesame seeds. After application, ethylene oxide dissipates by evaporation and reactions with matrix constituents. Aeration results in rapid decrease of ethylene oxide levels. The reaction products with food constituents include ethylene glycol, 2-chloroethanol and 2-bromoethanol (the latter two are formed in presence of chloride and bromide ions, respectively), and to a lesser extent diethylene glycol and dioxane. 2-chloroethanol may react with fatty acids, forming 2-chloroethanol-esters. Exposure of the consumer to ethylene oxide related residues through food will mainly concern the reaction products. The residue definition for ethylene oxide is the fumigant itself and 2-chloroethanol. In sesame seeds from the market, of these two compounds, only 2-chloroethanol is found at detectable levels. Ethylene oxide has hardly been monitored in food, and only a limited number of laboratories outside the EURL-NRL community had methods operational. Following the RASFF reports, the EURL and many other laboratories started implementation of existing laborious methods from the past, and/or development of new approaches such as QuOil modifications for sample preparation followed by GC-MS/MS analysis. Achieving the required limits of quantification is challenging. The EURL-SRM published a draft method on their website.
- The NRL Germany (BVL) reported on the 'German metabolite project', (same presentation as given in the EURL-FV workshop, see above).
- QA/QC. Same as in the EURL-FV workshop, see above.

EURL workshop AO [CVUA, Freiburg, Germany; 30 September-1 October 2020, online]

- *Presentation of EURL-proficiency test.* In 2020 a PT was organised for 108 pesticides (plus 2 voluntary) in rape seed oil. In total 123 laboratories participated. Nineteen pesticides were present, ranging from 0.03 to 0.19 mg/kg. Most pesticides were measured and reported by the majority (80-92%) of the laboratories, and in most cases quantitative results were satisfactory. The exception was spinosad, which consists of two components (spinosyn A and D). For the individual components only 40% of the participants reported satisfactory results. The reason was that many laboratories do not have the reference standards of the individual components which resulted in errors in quantification. The interlaboratory variability (RSD_R) varied from 13% to 48%, average 23% (slightly higher than fruit/vegetable and cereal matrices, see above).
- *Five technical presentations were given*. The EURL-AO presented the analysis methods they used for oil samples, which were either based on QuEChERS based (Qu-OIL) or GPC cleanup. Next, four NRLs presented their activities in pesticide residue analysis in general, and showed their analysis methods for samples of high fat/oil content: NRL Greece (BPI), NRL Croatia (CVI), NRL Netherlands (WFSR), and NRL Italy (ISS).
- *EU monitoring of infant formula and milk*. In 2019 samples were collected (78 and 54 respectively) by the NRLs from most EU member states and analysed by the EURL-AO. For infant formula a general 0.01 mg/kg MRL applies, and lower MRLs (0.003-0.006 mg/kg) for certain pesticides. The aim here was to measure at even lower levels (down to 0.0005 mg/kg) with emphasis on pesticides with low ADI values, in order to provide better data for risk assessment. Prioritised pesticides were chlorpyrifos, cyhalothrin, cypermethrin, fluquinconazole, and ethoprophos. Using adjusted multi-residue methods, this was possible for 46 out of the 59 targeted pesticides. In none of the samples pesticides were detected.
- The EURL-AO informed the NRL community on several activities from their working programme, which included a study on processing factors (dilution & cooking/baking) of animal products in processed foods (egg products, bakery products and sausages), and involvement in CEN standards (revision of EN 1528, pesticides/PCBs in fatty food).
- QA/QC. Same as in the EURL-FV workshop, see above.

8.1.2 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. A meeting was attended to discuss the results of proficiency tests organised by the EURLs in the first half of 2020, and to discuss comments received so far from the NRL/OL community on SANTE/12682/2019 (15-16 sept, online). WFSR also participated in the CEN working group on standardisation of methods for pesticide residue analysis (CEN/TC 275/WG3 Pesticides).

8.1.3 Participation in proficiency and comparative tests

The NRL participated in 29 proficiency tests on pesticides in fruits, vegetables, cereals, products of animal origin, feed and other food matrices. An overview is given in Table 8.1. A total of 277 z-scores (indicator for quantitative performance) were obtained. In general the performance of the NRL was satisfactory (268 out of 277) and the pesticides were correctly identified and quantified. For two pesticide/matrix combinations a questionable result was obtained (within statistically expected range). In seven cases the result was unsatisfactory, triggering a root cause analysis. In some cases a clear cause was identified (e.g. incidental ignoring correction for counter ions in concentration calculation), in other cases the cause remained unclear. Where appropriate, corrective actions were taken.

Table 8.1 *Overview of proficiency tests participation of NRL pesticides in food and feed*

PT Organiser and PT code	Matrix	Analytes[2)]	Performance [1)] S	Q	U
EUPT A015	Vegetable oil	MRM pesticides	19	0	0
EUPT CF14	Rice	MRM pesticides	19	0	0
EUPT FV22	Onion	MRM pesticides	19	0	0
EUPT SM12	Onion	MRM pesticides, screening	88% found		
EUPT SRM15	Onion	SRM pesticides (incl. chlormequat, glyphosate, paraquat, phenoxy acids)	15	0	0
Fapas 5141	Egg	MRM pesticides	6	0	0
Fapas 5143	Oily Fish	MRM pesticides	2	0	0
Fapas 5145	Milk powder	MRM pesticides	6	0	0
Fapas 5146	Pork fat	MRM pesticides	7	0	0
Fapas 5147	Olive Oil	MRM pesticides	2	0	0
Fapas 9128	Rice	MRM pesticides, bromide	11	0	0
Fapas 9129	Wheat	MRM pesticides	10	0	1
Fapas 9130	Oats	SRM pesticides (chlormequat, mepiquat, glyphosate)	3	0	0
Fapas 9133	Dry beans	MRM pesticides	9	1	1
Fapas 9134	Animal feed	MRM pesticides	7	0	0
Fapas 19281	Cabbage	MRM pesticides	10	1	0
Fapas 19285	Carrot	MRM pesticides	26	0	0
Fapas 19287	grapefruit	MRM pesticides	13	0	1
Fapas 19289	Mushroom	MRM pesticides; SRM pesticides (chlormequat, mepiquat)	11	0	1
Fapas 19290	Cucumber	MRM pesticides	13	0	0
Fapas 19292	Wine	MRM pesticides	10	0	0
Fapas 19295	Vine Leaves	MRM pesticides	12	0	0
Fapas 19296	Parsley	Dithiocarbamates	0	0	1
Fapas 19297	Spinach	MRM pesticides	12	0	0
Fapas 19299	Potato	MRM pesticides	12	0	0
Fapas 19300	Mango	Ethephon	1	0	0
Fapas 19303	Green tea	MRM pesticides	11	0	0
TestQual 125	Strawberry	Dithiocarbamates	1	0	0
TestQual 126	Pear	SRM pesticides (fosethyl, phosphonic acid)	1	0	2

[1)] number of pesticides in the PT and z-scores obtained: S= satisfactory, Q = questionable, U = unsatisfactory

[2)] MRM: pesticides amenable to multi-residue methods, target list typically around 200 pesticides.

8.2 Assistance to official laboratories

8.2.1 Quality control

In the Netherlands, besides WFSR, there is one laboratory performing part of the official analysis of dairy products. In the frame of a quality control program, one sample of milk powder (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 the Dutch NRL. Feedback by the NRL was provided to the OL.

8.2.2 Advice

No specific advice other than in the frame of the quality control mentioned under 8.2.1 was given.

8.3 Scientific and technical support to the competent authority

Input was provided on analytical options for determination of ethylene oxide and 2-chloroethanol within and outside WFSR to deal with the urgent need for sample analysis, following a RAFFS alert shortly after the summer of 2020 (see also 8.1.1 EURL workshop CF/SRM). Views on the need and possibilities for updating the scope of the analytical (multi)methods were exchanged (e.g. 1,4-dimethylnaphtalene, maleic hydrazide). For feed, results from a separate project on possible risks on non-compliances of materials imported from outside the EU were communicated.

8.4 Contacts with other NRL's

Contacts with other NRLs were through the EURL workshops and through a CEN meeting on pesticides (all online). WFSR participated in the EPRW 2020 (European Pesticide Residue Workshop, Granada, Spain, May 2020), which unfortunately had to be changed into an online event. Oral and poster presentations were shared as pdf. Due to COVID-19, other meetings and symposia were cancelled.

9 National Reference Laboratory Metals and Nitrogenous compounds in feed and food

Coordinator: Hanneke Brust

9.1 Activities within the EURL-NRL network

9.1.1 Participation in EURL-NRL workshops

The EURL for Metals and Nitrogenous compounds is hosted by the DTU in Denmark. In 2020, the EURL organised an online training on the determination of nitrite and nitrate and the annual EURL-NRL workshop.

The training on the determination of nitrite and nitrate in food and feed on June 18 focussed on the ion chromatography method with UV detection. Although the Dutch NRL uses a different analytical method for the determination of nitrite and nitrate (continuous flow analysis with spectrophotometric detection), there were useful discussions on several general analytical aspects such as sample preparation, stability and matrix effects and interferences.

The annual EURL workshop was held online on October 28 and 29. The meeting was attended by sixty-eight participants representing NRLs from each EU member state. The number of participants was significantly higher than previous years, most likely because the workshop was an online event this year.

Proficiency tests (PTs) organised by the EURL and issues associated with these PTs are usually extensively discussed during the workshop. In 2020, the EURL organised three different PTs:

- The first PT concerned the determination of total arsenic, cadmium, lead and total mercury (obligatory) and the optional compounds inorganic arsenic and methyl mercury in fresh frozen fish. A limited number of labs reported results for the optional compounds. In addition, the inorganic arsenic content in the PT sample was very low, yielding many results below the limit of quantification (LOQ). Evaluation of the performance of the laboratories on the analysis of inorganic arsenic was therefore not possible. For methyl mercury the statistic evaluation of the PT was for information only because of the limited number of results. The Dutch NRL did submit results for inorganic arsenic, but not for methyl mercury. Although the PT sample was analysed for methyl mercury, the analytical quality criteria were not met and hence the result was not reported.
- The second PT covered the determination of nickel, cadmium and lead, and optional the elements aluminium and copper in cocoa powder. The discussion focussed on aluminium, because of the large variation in the results submitted by the different laboratories, most likely due to incomplete extraction of aluminium from the matrix. Therefore a survey was sent to the NRLs on the analytical method that was used. The analytical methods were discussed and suggestions were made by the EURL to improve the analytical methods for aluminium.
- The third PT organised by the EURL concerned the determination of arsenic, cadmium, lead, mercury, nitrite and nitrate in the feed material molasses. The nitrite results could not be evaluated because the stability tests showed that nitrite was not stable in the PT material. The results of all compounds appeared to be skewed, which was attributed to results that were incorrectly reported by some labs. All results should have been corrected for the moisture content according to Directive 2002/32/EC, the nitrite content should have been expressed as sodium nitrite and nitrate as the nitrate ion. The EURL therefore requested the NRLs to check their results and to correct them if necessary shortly after the deadline of the PT. The Dutch NRL performed the moisture correction in the right way and therefore resubmission of the results was not necessary.

Other topics discussed at the EURL-NRL workshop included:

- *The Guidance document on the estimation of LOD and LOQ*. This guidance document, prepared in 2016 by four EURLs in the area of contaminants, is frequently used by the Dutch NRL during analytical method validation, but many labs consider the procedures described in the guidance document too complex and too laborious. The network of the four EURLs is therefore going to revise the guidance document. The importance of a proper determination of LOQs was also stressed during a presentation on the work performed by the EFSA contaminant panel. Low LOQs are required for exposure and risk assessment, and results from methods with high LOQs are all discarded. This has for example been an issue for nickel data that was collected since 2015 in order to update the EFSA Opinion on nickel in food and drinking water.
- *News from the Commission*. DG SANTE presented recent and future developments of the regulations for metals and nitrogenous compounds in feed and food. Discussions are ongoing to revise several maximum limits (MLs). Plans to lower several MLs in food (e.g. cadmium in vegetables and fruit, lead in foods for infants) stressed again the importance of low LOQs and proper determination of the LOQs. Based on occurrence data collected by EFSA the past few years, the Commission is considering to establish maximum limits for nickel in feed and arsenic in different foodstuffs (in addition to rice). The Commission planned to publish a recommendation on monitoring of inorganic arsenic in additional feed materials in 2020, but this was delayed by more than a year and is now expected to be published in 2021.
- *Analysis of nitrite and nitrate*. Nitrogenous compounds have been added to the scope of the EURL in 2019, and therefore the analysis of nitrite and nitrate is still relatively new for some NRLs. A presentation on the determination of nitrite and nitrate was therefore given by the EURL. A major issue with the determination of nitrite is its stability, which was studied by the EURL. Nitrite appears to be unstable at low pH and in the presence of different metals. The instability of nitrite was also the reason that it was not possible to evaluate the nitrite results of the third EURL PT. In addition, an overview of analytical methods for the determination of nitrite and nitrate was presented.

9.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to metals or nitrogenous compounds in food and feed in 2020 to participate in.

9.1.3 Participation in proficiency and comparative tests

The NRL has participated in three proficiency tests organised by the EURL. In addition, the NRL participated in several other internationally organised PTs (Table 9.1).

All results of the reported concentrations in the PTs mentioned in Table 9.1 were satisfactory (z-scores between -2 and +2), except for mercury in mushroom powder (FAPAS 07381). The z-score of 2.8 was attributed to a matrix effect. Although WFSR as official laboratory did not routinely analyse mushrooms in the past years and therefore the unsatisfactory z-score did not affect previously reported results in routine samples, the method was adjusted to prevent possible issues in the future.

Table 9.1 *Overview of proficiency and comparative tests, NRL metals and nitrogenous compounds*

PT	Analytes	Matrix	z-scores
FAPAS 07353	As, Cd, Pb, Hg, Ni	Mixed corn (feed)	Between -1.1 and 0.2
FAPAS 07366	Al, As, Cd, Cu, Pb, Hg, Ni, Zn	Wheat flour	Between -0.3 and 1.5
FAPAS 07374	Ni, Cd, Pb	Herb (mixed, dried)	Between 0.3 and 0.9
FAPAS 07381	Ni, As, Cd, Pb, Hg	Mushroom powder	Between -0.4 and 0.9; *2.8 for Hg*
EURL-MN PT-2020-01	As, Cd, Pb, Hg, iAs	Fresh frozen fish	Between -0.18 and 0.40
EURL-MN PT-2020-02	Al, Ni, Cu, Cd, Pb	Cocoa powder	Between -0.56 and 0.72
EURL-MN PT-2020-03	As, Cd, Pb, Hg, nitrate (NO_3)	Molasses (feed)	Between -0.65 and 1.56
FAPAS 30100	Melamine, cyanuric acid	Infant formula	0.2 and -0.7, resp.
FAPAS 15145	Nitrate (NO_3)	Cabbage puree	0.0
FAPAS 15144	Nitrate (as $NaNO_3$) and nitrite (as $NaNO_2$)	Meat	-0.2 and 1.4, resp.

9.2 Assistance to official laboratories

9.2.1 Quality control

Analyses for the competent authority (CA) for milk and milk products are carried out by an OL. The CA revised the list of official analyses in the context of Regulation (EU) 2017/625. The revised list includes analysis of heavy metals, minerals and trace elements, and nitrite and nitrate. The quality of the OL assigned by the CA for these analyses was investigated by the Dutch NRL. This investigation included a test round with samples with a known metal content, CRM materials or spiked samples that were sent to the OL and routine samples from the OL that were reanalysed by the NRL. The results of the analyses by the OL were discussed with the CA milk and milk products and reported.

9.2.2 Advice

Advising the OL for milk and milk products is on ad hoc basis and depends on the outcome of the comparison test organised two times a year by the NRL. In 2020 no specific advice was given based on the comparison test.

9.3 Scientific and technical support to the competent authority

WFSR provided information to the competent authority on arsenic speciation in fish samples analysed for the National Plan Residues. A threshold level of total arsenic was discussed, above this threshold samples will be analysed for the inorganic arsenic content in the coming year (2021).

9.4 Contacts with other NRL's

There were contacts with other NRLs through the EURL workshops and through the CEN meeting of the working group on elements and their chemical species. The report of a collaborative trial, organised by the German NRL in 2019, on a method for mercury in food was published this year and the results and the report were discussed with the German NRL.

10 National Reference Laboratory Mycotoxins and Plant toxins in feed and food

Coordinator: Hans Mol

10.1 Activities within the EURL-NRL network

10.1.1 Participation in EURL-NRL workshops

In 2020 a workshop by the EURL for mycotoxins and plant toxins in feed and food (WFSR, Netherlands) was held on 6-7 October. Due to COVID-19 related travel and meeting restrictions, the workshop was held as online meeting. Seventy one 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, standardisation) 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 legal and emerging issues, by the Directorate-General for Health & Food Safety (DG SANTE). 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*) has been lowered from 2,000 µg/kg to 100 µg/kg. 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 1.7.2023, 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 1.7.2023. For cereal-based baby food, a fixed maximum level of 20 µg/kg is proposed. For deoxynivalenol (DON) in both food and feed, the inclusion of three other forms (3-acetyl-DON, 15-acetyl-DON, DON-3-glucoside) in the maximum levels is considered premature for now. On the other hand, the existing MLs for DON might be lowered by some 20% based on recent occurrence data and following the ALARA principle. For T-2 and HT-2 toxin the currently existing indicative levels will change to generally lower maximum levels (20-50 µg/kg for the sum in cereal grains for the final consumer, higher for unprocessed cereals. 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, bread cereal snacks, mostly in the 1.5-10 µg/kg range). 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 maximum levels, but in case of repetitive exceedances, investigations should be performed to find out the cause and possibilities to reduce the contamination. For feed changing of the current guidance values for some mycotoxins in Recommendation 2006/576/EC into (slightly lower) maximum levels in the frame of Directive 2002/32/EC is considered. With the Annexes of Directive 96/23/EC being repealed as from 14/12/2022, mycotoxins (and plant toxins) in products of animal origin will be included in the scope of the EURL Mycotoxins and Plant toxins.
- Plant toxins. For erucic acid, adjusted maximum 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 being finalised on maximum levels in food (herbal infusions (dried product), dried herbs, food supplements) 200-400 µg/kg for the sum of PAs (up to 1000 µg/kg for certain herbs). For tea (*Camellia sinensis*) this is 150 µg/kg. Also there appears to be consensus on which PAs need to be included in the sum of PAs: 21 representative marker PAs plus 14 PAs that cannot always be distinguished from these 21. For tropane alkaloids (atropine, scopolamine) maximum levels for baby food will apply to a wider range of ingredients, and maximum levels will also be set for millet, sorghum, maize, buckwheat and herbal infusions. For opium alkaloids maximum levels are considered (morphine equivalents = morphine + 0.2x concentration codeine) of 20 mg/kg poppy seed and 1.5 mg/kg for bakery products containing poppy seeds. 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 (unprocessed linseed, 150 µg/kg, almonds, 20 µg/kg, and cassava flour, 10 µg/kg). Possible maximum levels of THC (delta-9-tetrahydrocannabinol) in hemp-derived food in the range 3-7.5 mg/kg are under consideration. For feed, the current maximum level of 1 gram of seeds of Datura sp might not be protective enough for horses and pigs, adjustments are considered.

- *A technical presentation* was given by the Dutch NRL on the recently published EFSA opinions on glycoalkaloids, quinolizidine alkaloids, and opium alkaloids, with emphasis on analytical aspects (methods, availability/stability of reference standards).
- *Discussion of EURL-proficiency tests (PTs).* A follow-up presentation was given on the outcome of an interlaboratory research study on pyrrolizidine alkaloids organised in 2019, taking the current consensus regarding inclusion of 35 PAs into account. Quantification of PA isomers as group when separation is not possible appeared the best approach to achieve comparable results. Next, results were presented for the PT organised end 2019 on ergot alkaloids in rye and a cereal mix. Despite some analytical difficulties (epimer conversion, relatively short experience for many laboratories), the results were mostly satisfactory. For results of the Dutch NRL in these PTs see below.
- *Method performance criteria.* Following a first discussion on new and more generic method performance criteria at the 2019 workshop, the Analytical Quality Control group consisting of EURL and NRL representatives, drafted a revision of the relevant annex in Regulation (EC) No 401/2006, and a background guidance document. The feedback received from the NRL community was discussed and consensus was reached on most definitions and criteria (a.o. LOQ, recovery, RSDs).
- *Miscellaneous.* To inform the NRL community, an update of activities within CEN for mycotoxins and plant toxins in food was given by a representatives of the NVWA and in feed by a representative of WFSR. Nestlé presented the results from an extensive interlaboratory validation for multi-mycotoxins methods in various food products, organised within CEN. The CEN standards developed under mandate 520 are in the final stage. Topics for a new standardisation request are being inventoried.
- *EURL working program 2021*, will include training activities on analytical methods, and PTs on determination of multiple mycotoxins in the same sample with emphasis on multi-mycotoxins methods, and possibly opium alkaloids.

10.1.2 Participation in working groups

As part of the EURL task, WFSR has initiated a working group on analytical quality control (AQC) in which NRL representatives participate. No meetings were held in 2020. Discussions were online by commenting on various revisions of a guidance document drafted by the EURL.

10.1.3 Participation in proficiency and comparative tests

The NRL participated in 18 proficiency tests, all on mycotoxins. An overview is given in Table 10.1. In general the performance of the NRL was satisfactory and the toxins were correctly identified and quantified. For three out of 84 toxin/matrix combinations a questionable result was obtained (within statistically expected range), and one unsatisfactory result. The latter concerned aflatoxin B2 and did not affect the result for the sum of aflatoxins.

Table 10.1 *Overview of proficiency tests participation of NRL mycotoxins and plant toxins*

PT Organiser and PT code	Matrix	Analytes[2)]	Performance [1)]		
			S	Q	U
FAPAS 1672	apple juice	Patuline	1	0	0
FAPAS 1673	apple sauce	Patuline	1	0	0
FAPAS 4375	Animal feed	aflatoxin B1, ZEN, DON	3	0	0
FAPAS 4380	black pepper	aflatoxins, OTA	6	0	0
FAPAS 4382	Oats	aflatoxins, OTA, T2	6	0	1
FAPAS 4383	Animal feed	Aflatoxins	5	0	0
FAPAS 4385	Babyfood	Aflatoxins	2	0	0
FAPAS 4386	almond	Aflatoxins	4	1	0
FAPAS 4389	Paprika powder	aflatoxins, OTA	6	0	0
FAPAS 4394	Chili powder	aflatoxins, OTA	6	0	0
FAPAS 4395	Peanut	Aflatoxins	5	0	0
FAPAS 4396	Milk Powder	Aflatoxin M1			
FAPAS 4400	Rice	Aflatoxins	5	0	0
FAPAS 22167	Breakfast cereals	DON, ZEN	2	0	0
FAPAS 22169	Rye	ergot alkaloids	4	1	0
FAPAS 22174	Baby Food	ergot alkaloids	12	0	0
FAPAS 22175	Animal feed	DON, ZEN, T2, HT2	5	0	0
PROGRETTO MA2050	Maize	aflatoxins, OTA, FB, DON, ZEN	7	1	0

[1)] number of toxins in the PT: S= satisfactory, Q = questionable, U = unsatisfactory.

[2)] OTA = ochratoxin A, DON = deoxynivalenol, FB = fumonisins, ZEN = zearalenone.

10.2 Assistance to official laboratories

10.2.1 Quality control

Besides the NRL that also acts as OL, there is one other OL in The Netherlands. 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.

10.2.2 Advice

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

10.3 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 2020 there was specific exchange of information regarding THC and CBD in CBD oils used as food supplement.

10.4 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 (all online). Due to COVID-19, other meetings and symposia were cancelled.

11 National Reference Laboratory for Processing Contaminants

Coordinator: Liz Leenders

11.1 Activities within the EURL-NRL network

11.1.1 Participation in EURL-NRL workshops

The EURL for Processing contaminants (PC) is hosted by the DTU in Denmark. In 2020, the Dutch NRL participated in the annual workshop of the EURL hosted in two online sessions on September 29 and 30. 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. The first online session was focused on an evaluation of all PTs organised by the EURL in 2020, followed by some interesting presentations regarding the analysis of processing contaminants in a variety of food products. The second session focused on presentations regarding some different techniques for the analysis of processing contaminants, followed by an update from the Commission, and discussions about the suggested performance criteria for methods of analysis of processing contaminants by the EURL.

- *Discussion of the EURL PTs in 2020*. The workshop started with a discussion on the three PTs that were organised by the EURL in 2020. The first PT concerned the determination of 2- and 3-monochloroprane-1,2-diol (MCPD) esters and glycidyl esters (GE) in infant formula. Several laboratories reported unsatisfactory scores for glycidyl esters. This was attributed to the fact that glycidyl esters did not pass the stability tests, so the z-scores were only for indicative purposes. The next PT concerned the determination of furans in baby food. Since furans is a relatively new subject, not all NRLs participated in this PT. Three laboratories reported extremely high concentrations of furans, which increased the robust mean value for these analytes significantly. One NRL therefore suggested that the EURL should check if these three labs could be considered to be outliers and therefore could be eliminated from the statistical evaluation. Overall, for furan, 2-methylfuran and 3-methylfuran, only half of the participating laboratories scored acceptable z-scores. Reporting a concentration for 2,5-dimethylfuran was on voluntary basis, but since only eleven laboratories reported results, the results could not be assessed. The last PT concerned the determination of acrylamide in baby food. Out of the 36 laboratories participating in this PT, only one laboratory got a questionable result. Lastly, the EURL-PC discussed the future PTs and other activities within the network for 2021, which included PTs of polycyclic aromatic hydrocarbons (PAHs) in plant product and 3-MCPD and glycidyl esters in infant formula, and a training for PAH analysis of plant products, spices and food supplements.
- *Analysis of processing contaminants in a variety of food products*. Three EURL representatives gave interesting presentations regarding the analysis of processing contaminants in a variety of food products. The EURL shared the challenges in estimation of PAH exposure from barbecuing. The EURL shared their analytical method for the analysis of MCPD, GE and acrylamide in vegetable crisps and their research on acrylamide in vegetable fries other than potatoes. The conclusion in these last presentations was that due to the fact that vegetable crisps contained higher levels of MCPD, GE and especially acrylamide than potato crisps, consumption of vegetable crisps leads to higher health risks related to dietary acrylamide intake than potato crisps consumption. The acrylamide concentration in homemade vegetable fries made from sweet potato and carrot and the current consumption of potato fries is so high that it may result in health risk to the consumers as acrylamide is a genotoxic and carcinogenic substance.
- *Updates from the Commission*. DG SANTE presented recent and future developments of the EU policy and regulations for processing contaminants. Starting in 2021, new maximum levels regarding glycidyl esters in fish oil and infant formula, as well as maximum levels for the sum of free 3-MCPD and 3-MCPD esters will apply. An overview of all food and feed safety alerts (RASFF) notifications

was shown and some alerts on the list were discussed. Future work regarding acrylamide was discussed. The existing benchmark levels in Regulation (EU) 2017/2158 will be revised in view of lowering them to reflect the continuous reduction of the presence of acrylamide. Furthermore, benchmark levels will be established for foodstuff categories not covered by the current Regulation. Maximum levels (MLs) for acrylamide will be set. As a first step MLs are considered in biscuits and baby food for infants and young children. New MLs for PAH in traditionally smoked meat and fish and in plant powders were presented. Furthermore, a draft recommendation for monitoring of furan and methylfurans was presented. EFSA has identified a health concern related to the presence of furan and methylfurans in food. There are however not sufficient occurrence data on methylfurans in certain foods to perform a reliable exposure assessment. The draft recommendation foresees to generate these data for a variety of food products in view of possible future risk management measures to protect public health. Lastly, the Commission mentioned that EFSA has been asked for a scientific opinion on the risks for human health related to the presence of N-nitrosamines in food, with a deadline in 2022.

- *Analysis of processing contaminants in food using different techniques.* Two NRL representatives gave interesting presentations on different techniques for the analysis of processing contaminants out of scope of the EURL-PC. NRL Germany (BVL) shared the analysis of furan and alkylfurans in baby food by sample enrichment in the cold injection system (CIS) as an alternative to solid phase microextraction-gas chromatography- mass spectrometry (SPME GC-MS). The EURL-PC shared the analysis of 5-hydroxymethylfurfural in caramel coloured cookies by high performance liquid chromatography – ultra violet spectroscopy (HPLC-UV).
- *Discussion on the suggested performance criteria*. Within the NRL network the analytical method performance criteria described in the EU legislation have been discussed. The performance criteria for each of the processing contaminants are described differently, given challenge to the NRLs and OLs when validating the analytical methods. Furthermore, some of the laboratories suggested that some of the performance criteria were too strict for routine analysis. The EURL made some suggestions to change these performance criteria set by EU Recommendation for all processing contaminants. The workshop was concluded with an interactive session in which the NRLs discussed the suggested performance criteria for processing contaminants by the EURL.

11.1.2 Participation in working groups

There were no working groups on EURL-NRL issues related to processing contaminants in food in 2020 to participate in. The EURL is discussing the installation of such focussed working groups in the future.

11.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL includes PAHs, 2- and 3-MCPD esters and GE esters, acrylamide and furan/methylfurans. The Dutch NRL participated in PTs for these processing contaminants. In 2020 the NRL participated in three PTs organised by the EURL. In addition, the NRL participated in several other international PTs organised by other organisations as shown in Table 11.1.

Table 11.1 *Overview of proficiency tests, NRL processing contaminants*

PT	Analytes	Matrix	z-scores
EURL 2020-04	2- and 3-MCPD esters and glycidyl esters	Infant formula	Between 0.8 and 2.3
EURL 2020-05	Acrylamide	Baby food	0.7
EURL 2020-05	Furan, 2-methylfuran, 3-methylfuran and 2,5-dimethylfuran	Baby food	Between -1.8 and -2.0
FAPAS 2660	2- and 3-MCPD esters and glycidyl esters	Potato Crisps	Between 0.1 and 1.6
FAPAS 2661	2- and 3-MCPD esters and glycidyl esters	Infant formula	Between 0.4 and 1.0
FAPAS 2662	2- and 3-MCPD esters and glycidyl esters	Vegetable oil	Between 0.2 and 1.2
FAPAS 30101	Acrylamide	Vegetable Crisps	0.1
FAPAS 30102	Acrylamide	Infant food	-0.4
FAPAS 30104	Acrylamide	Biscuits (Cookies)	0.3
FAPAS 0695	PAH	Spirulina powder	Between 0.0 and 1.6

The majority of the results mentioned in the table above were satisfactory (z-scores between -2 and 2), yet there was one result that was > 2. This unsatisfactory result was a z-score of 2.3 for glycidyl esters in the EURL PT. However, the EURL stated that this z-score was for indicative purpose only since the samples did not pass the stability test for glycidyl esters, so this factor might have influenced the reported results of the participating laboratories. Also, in the PT for furan/methylfurans in baby food organised by the EURL, no z-score was assigned for 3-methylfuran since the measured value by WFSR was reported to be below LOQ. The assigned value reported by the EURL-PC was above our LOQ, so in the concentration of 3-methylfuran should have been measurable. Overall, all z-scores received in this PT were negative values (z-scores 0 < -2). Due to wrong storage of the sample at WFSR the glass package of the baby food exploded, leading to loss of furans due to their volatility. This loss of furans lead to lower measured values in the baby food, resulting in these negative z-scores and the lower value for 3-methylfuran (<LOQ).

11.2 Assistance to official laboratories

11.2.1 Quality control

The laboratories of the OL and the NRL merged in 2019, both former separate laboratories participated in the PT for PAHs organised by FAPAS. The z-scores were evaluated by the NRL. Quality assurance was established by confirmation of PAHs concentrations in several samples analysed by the former OL.

11.2.2 Advice

Since the merger of the laboratories of the OL and the NRL, advice on analytical measurements, quality and measurement strategies are given on a regular basis.

11.3 Scientific and technical support to the competent authority

In 2020, there have been several contact moments between the NRL and the ministries of Agriculture, Nature and Food Quality (LNV), of Health, Welfare and Sport (VWS), NVWA and RIVM with regards to processing contaminants in food. Furthermore, a report was published regarding a PT on PAHs in meat organised by WFSR in 2019.

11.4 Contacts with other NRL’s

During the online EURL workshop 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.

12 National Reference Laboratory for halogenated persistent organic pollutants (POPs)

Coordinator: Caroline Dirks

12.1 Activities within the EURL-NRL network

12.1.1 Participation in EURL-NRL workshops

In 2020 two EURL-NRL online workshops have been held. First a one day workshop on the 19th of May and thereafter a two day workshop from 17-18 November.

The representative of the Directorate-General for Health and Food Safety of the EU Commission (EC) gave an update on (regulatory) issues in regards to POPs in feed and food at EU level at both workshops. These included:

Notifications
A summary of RASFF notifications related to dioxins, dioxin-like polychlorinated biphenylethers (dl-PCBs) and non-dioxin-like PCBs (ndl-PCBs) since the last EURL/NRL meeting in November 2019 was given:

- The first notification was related to copper chelate from the Netherlands with an elevated level of dioxins.
- The second notification was on ndl-PCBs in feed premixture from Spain. Further investigation of the second case showed connections of the contamination with the use of oleoresins from capsicum plants for colouring. The patterns were primarily dominated by PCB 28 and, to a lesser degree, PCB 52.
- Other notifications in feed were on Polychlorinated dibenzodioxins/furans (PCDD)/Fs in dog chews from India, two cases of dioxins (PCDD/Fs) in zinc oxide from Germany without elevated PCB levels and one notification on PCDD/Fs and dl-PCBs in palm fatty acid distillate from the Netherlands. This last notification was remarkable, as no non-compliant levels were reported – merely the results of an investigation into suspected contaminant dilution following a client complaint in the Netherlands.
- Notifications in food were on PCDD/Fs in chilled boneless beef from Japan, PCDD/Fs in vegetable stearic acid food grade E570 from Germany and PCDD/Fs in eggs from Germany. In the last notification the soil of a hen farm was determined as the source of the egg contamination.

EFSA opinions
The following EFSA opinions related to the field of halogenated POPs were either adopted, are in the process of adoption or are currently ongoing:

- Chlorinated paraffins (CPs): The final version of the Opinion was adopted in February 2020. CPs are still considered of some concern by the EC, but there is no immediate need for action. Further steps are waiting on method development/ harmonisation by the EURL, afterwards followed by a monitoring project.
- Per- and polyfluoroalkyl substances (PFAS): The final opinion was adopted on 17 September 2020. Evaluating the 4 PFASs: perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and perfluorononanoic acid (PFNA), a group Tolerable Weekly Intake (TWI) of 4.4 ng/kg bw/week was established. Drinking water, fish, fruit, eggs and egg products were identified as critical sources. Regulatory follow-up by the EC has started in September with a special focus on analytical performance in food and feed. Other remarks made by the EC on PFAS are:
  - As a high level of carry-over of PFAS from hen's feed to eggs has been reported, setting up a feed monitoring programme might be possible.

- Under the chemical strategy for sustainability, the EC will ban all PFASs in fire-fighting foams and other uses in the EU, with exceptions only for products that are 'essential for society'. PFASs will be addressed with a group approach, meaning more than one substance will be included in maximum levels (MLs), under the relevant legislation for water, food, emissions, waste and sustainable products.

- *Brominated contaminants*: Work on Hexabromocyclododecane (HBCDDs) tetrabromobisphenol A (TBBPA), BPh, polybrominated diphenyl ethers (PBDEs) and similar compounds are currently ongoing. Additionally, the EC formally requested an update of EFSA's risk assessment of fish consumption.
- *Lowering of maximum Levels (MLs) PCDD/Fs and PCBs*: A revision of the current WHO-TEF-values (especially concerning PCB 126) was requested, finalisation is planned for the end of 2022. The review of current ML and Action Level (AL) based on available occurrence data indicates scope for very significant reductions in some cases. The EURL is investigating analytical feasibility of these reductions. Due to the delay of the revision of WHO-TEF values, the comprehensive review of ML and AL has been postponed until 2023. Instead, smaller quick-fixes/limited reviews of certain MLs will take place in the meantime if necessary. As a result of these discussion the EURL performed analytical tests to evaluate if the proposed lower MLs are achievable by routine methods without substantial changes to the current analytical methods. In accordance with the Regulation (EU) 2017/644 for food and Regulation (EC) No 152/2009 (as amended by Regulation (EU) 2017/771) for feed the targeted LOQ should be one fifth of the new MLs. The mean of LOQs of WHO-PCDD/F-TEQ reported by the NRLs in the questionnaire of January 2019 was also used for comparison. The EURL concluded:
  - Feed: For feed matrices of plant and mineral origin the target LOQs are achievable by increasing the sample weight to 20 or even 30 g. However, for feed matrices with a high lipid content the target LOQs could not be achieved because a sample intake above 3 grams of fat would need scaling up of the clean-up steps and thus changing the analysis method.
  - Food: for egg and milk the target LOQs are achievable by increasing the sample weight to 5 grams. This was not the case for pork, for this matrix also the increase of sample weight would mean scaling up of the clean-up steps and thus changing the analysis method.

Addition of a new group of POPs; the polychlorinated naphtalenes (PCNs). The EURL has been setting up a method for this group of substances in the past year and is now waiting for the outcome of the EFSA risk assessment. Depending on the outcome the EURL needs to actively assist NRLs in setting up methods in the upcoming working programme period (2021/22).

LOD/LOQ Guidance Document: At the cluster meeting of four EURLs it was concluded to write one joint document including one recommended and generally described approach on how to estimate LODs and LOQs. The document will include an annex with practical examples from all fields of analysis. Other approaches for LOD/LOQ estimation will only be included for sound reasons, e.g. if required by legislation, in case of high blank levels or if the method is firmly established for certain analytes.

The results of the PTs for Fish Fillet (prepared from regular market fish) and Feed were discussed.

Update on the EFSA PFAS opinion by a representative of the CONTAM panel of EFSA
In the new EFSA opinion mixtures of PFASs generally are taken into account but the focus is on the 4 PFASs: PFOA, PFNA, PFHxS and PFOS. A TWI of 4.4 ng/kg bw/week was derived for the sum of the four PFASs, which is lower than the combined TWIs from the former EFSA opinion, from 2018. Ninety percent of the data on the occurrence of PFASs that was reported by the member states was below limit of detection (LOD). This means that for the exposure assessment calculations the upperbound (LOQ) values were used. However this may have led to an overestimation of the exposure because of the high LOQs.

The food groups contributing most to the exposure for PFOS and PFOA are besides fish with the highest contribution, eggs, fruits, vegetables and drinking water. For further evaluation more data in regularly consumed food with lower LOQs, in critical food groups and on the contribution of other

PFASs is necessary. Additionally, other analytical options like methods to measure total PFASs or specific fragments or total effects in bioassays need to be considered.

The Dutch NRL for halogenated POP's participated in 3 core working groups (CWG): PFASs, chlorinated paraffins (CPs) and brominated flame retardants (BFRs).

An update was given on the activities of the Core Working Group for PFAS
Activities organised by the CWG PFAS in 2019/2020 were the EURL training course on analysis of PFAS (November 2019), the EURL PT on determination of halogenated POPs in fish and the amendment of the LOD/LOQ guidance document with regards to PFASs. Reference was made to the EFSA opinion as to amend the present 'mandatory' spectrum of two PFASs to the four PFASs of the new opinion of EFSA (see xx).

A questionnaire was sent by the CWG to the members of the CWG to learn more about the capabilities of laboratories analysing PFASs. For internal laboratory performance control a PFAS QC laboratory material (freeze-dried fish) was provided by Örebro University. Twelve laboratories, including the Dutch NRL, expressed interest in the analysis of the sample and have received it through the EURL. The results will be discussed at the next meeting of the CWG in June 2021.

The CWG PFAS furthermore drafted an analytical guidance document for the analysis of PFASs in food based on the evaluation of the questionnaires mentioned above. It was agreed that the focus will be on the four mandatory PFASs: PFOS, PFOA, PFHxS and PFNA.

The chair of the CWG PFAS ended to act as chair but is willing to support the work of the CWG PFAS as an expert in the future. An expert from WFSR has taken over this task.

An update on the activities of the Core Working Group for brominated flame retardants (BFR)
An updated version of the guidance document on analytical parameters for the determination of organobromine contaminants in food and feed was distributed within the network and commented.

A draft annex to this guidance document with practical examples of methodology was made, this annex initially will focus on PBDEs and will later be extended with methodologies for HBCDDs. The annex is intended as a starting point for laboratories new to PBDE analysis and will include among others practical information on standards, on sample preparation and reporting. Additionally, the annex uses a modular approach to describe manual and automated extraction and clean-up methods and different measurement techniques, in order to allow for flexibility so that laboratories can chose what suits them best. These six to seven modules can be combined individually depending on the laboratory equipment.

The EURL training course on brominated contaminants, in particular PBDEs and HBCDDs was held on the 4th of November 2020 as webinar and, therefore, was without practical laboratory demonstrations this time. It was a very interactive training with 11 participants, most of them shared their experiences and issues via presentations. A practical session is planned for the future.

In general, the number of laboratories participating in Proficiency Tests (PTs) for PBDEs and HBCDDs is increasing as seen in the last PTs and also the results of the laboratories are very encouraging. For emerging brominated contaminants in the current EURL PTs 2020 (fish fillet and feed fats) results for seven analytes of the CWG *initial list of emerging BCons* were given including TBBPA (tetrabromobisphenol A), DBDPE (decabromodiphenylethane), 2,4,6-Tribromophenol, PBEB (pentabromoethylbenzene), HBB (hexabromobenzene), PBBz (pentabromobenzene) and PBT (pentabromotoluene). Nevertheless, more data is needed for a reliable comparison of participants' results and methods on emerging BCons.

Another important activity of the CWG is the surveillance of findings in new literature and these findings have provided support for the initial list of emerging brominated contaminants. As new data and information, e.g. toxicology, occurrence etc., becomes available, the initial list of emerging brominated contaminants may be modified accordingly.

At the CWG meeting it was decided that the aim will be to lower the LOQ's for PBDEs to 0.001 ug/kg fresh weight except for BDE209.

An update on the activities of the Core Working Group for chlorinated paraffins (CPs)
The CWG CP has finalised a first version of the main guidance document on CP analysis. The document, which was also distributed to all NRLs, includes the following sections:

- Definition of analytes
- Methods of sample preparation
- General categorisation of instruments
- Advice on suitable standards
- Comparison of some quantification methods
- Reporting requirements
- Proposed QA/QC measures
- Annexes with examples of methods

The annexes addressing possible analytical criteria and methodologies for simplified CP quantification are in preparation. It was agreed that especially for the analytical criteria more experimental and performance data are needed. The CWG is therefore collecting sample measurement data including data on the methods used in a database for further evaluation.

Concerning an advice on suitable standards for CP analysis, a statement letter was drafted and discussed. Considering the complexity and breadth of issues, it was decided to form a Core-CWG CP standards in which WFSR is participating, tasked with writing a scientific publication presenting the CWGs position on standards to ensure maximum impact and dissemination. The first meeting of the CCWG CP standards was held in December 2020, and a first draft version of the publication is planned for presentation on the next CWG meeting in March 2021.

12.1.2 Participation in proficiency and comparative tests

In 2020 the Dutch NRL participated in four PTs organised by the EURL and one PT that was organised by FAPAS. The results are summarized in the Table 12.1 below.

Table 12.1 *Results in PTs*

Name of PT	Topic	z-score
FAPAS	Perfluorinated Alkyl Substances (PFASs) in salmon	0.7
EURL	Perfluorinated Alkyl Substances (PFASs) in Fish Fillet (fresh water)	1.5 (PFOS) – 3.3 (PFUnDA)
EURL	PCDD/Fs, PCBs, PBDEs and BFRs in Fish fillet	-0.6 to 2.9
EURL	PCDD/Fs, PCBs, PBDEs and BFRs in Feed	-0.8 to 2.2
EURL	Chlorinated paraffins in Fish Fillet	-0.6 to 1.2

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 (table above). The unsatisfactory results were: PT from the EURL (Fish Fillet) which was analysed for PFASs, the results for PFDA and PFUnDA were > 2 while for 12 PFASs no assigned value could be calculated because the results differed too much, due to very low amounts around the LOQ. The unsatisfactory z-score results for the PFAS' could partly be solved by using more accurate pipettes and separate standards for the different congeners instead of purchasing a standard mixture.

The same PT sample was also analysed for PCDD/Fs and PCBs with deviating results for PCB 123 (z-score 2.1) and 1,2,3,4,7,8-HxCDF (z-score 2.9), both of which don't contribute much to the final TEQ value. A PT in feed from the EURL (Rapeseed Oil) for PCDD/Fs and PCBs had 1 unsatisfactory z-score namely for 1,2,3,4,6,7,8,9-OCDD (z-score 2.2). This however had no consequences for the sum TEQ values. For the few individual PCDDs/Fs with high z-score no particular solution was found after discussion with the EURL. Moreover the contribution of these congeners to the sum toxic equivalency quotient (TEQ) are negligible so no further action has been undertaken. As for the

PCB 123, this congener should have been reported as < LOQ, this error has been discussed with the lab technicians.

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2020 four dairy samples were sent to the OL for analysis on PCDD/Fs and PCBs. The results were satisfactory, although a slight negative bias was observed. The quantification limits used by the OL are considered to be sufficiently low. The reproducibility of the results for the WHO-PCDD / F-PCB sum-TEQ meets the statutory requirement of 25% (Regulation (EU) 2017/644).This was reported back to the OL.

12.2.2 Advice

No particular advice has been exchanged with the OL.

12.3 Scientific and technical support to the competent authority

There has been support of the Ministry of Agriculture, Nature and Food Quality, Ministry of Public Health related to items on the agenda of the European Commission and EFSA Opinions, as well as questions about POPs in food and feed.

12.4 Contacts with other NRL's

The NRL has been in contact with the EURL and other NRLs to share data on standards that have recently been put on the market for the quantification of chlorinated paraffins. The result of this cooperation is written down in a scientific paper that will be published in 2021.

13 National Reference Laboratory Food Borne Viruses

Coordinator: Ingeborg Boxman

13.1 Activities within the EURL-NRL network

13.1.1 Participation in EURL-NRL workshops

The EURL-NRL network foodborne viruses was started in 2018. The focus of this network is implementing analyses of food for the presence of norovirus (as cause of gastroenteritis) and hepatis A virus (as cause of hepatitis A) using the ISO 15216-1:2017. The EURL is based in Uppsala at the Swedish Food Safety Agency. WFSR was appointed as the NRL for foodborne viruses. RIVM has been appointed as NRL for foodborne viruses in the specific matrix of bivalve molluscs as having hosted the former NRL bivalve molluscs. Knowledge and expertise on this matrix is also present at WFSR, as WFSR has analysed the presence of norovirus and hepatitis A virus under accreditation in this matrix for over 10 years.

The NRL participated in the 3rd workshop of the EURL-NRL for foodborne Viruses. This meeting was hosted by the EURL (the Swedish Food Safety Agency) via a web conference, September 1-3, 2020. The meeting was joined by 43 participants from many countries. In 2020, the NRL network welcomed the newly appointed NRLs from Austria, Cyprus, Luxembourg, Slovenia and Poland.

During the meeting the former PTs (results) and future PT schemes were discussed. It was decided that PT schemes should cover at least bivalves once a year, but also seek the possibility to include, besides soft fruit and lettuce, also bottled water and food surfaces that are described in the ISO 15216.

The NRL network agreed with an initiative of WFSR, to include the possibility for testing reverse-transcription digital polymerase chain reaction (RT-dPCR) in the next PT for virus-contaminated oysters. WFSR was appointed project leader of this comparison study in cooperation with the EURL. The outcome of the study will potentially provide results that will lead to more solid conclusions regarding the future direction of the use of RT-dPCR as an alternative to reverse-transcription real-time polymerase chain reaction (RT-qPCR).

Much attention was given to several aspects of norovirus in bivalve molluscs, including a discussion whether there is a 'safe' maximum viral contamination level, and the technical issues encountered (e.g.no discrimination between infectious or non-infectious particles, measurement uncertainties). An EFSA representative shared outcomes of the recent EFSA baseline study on the presence of norovirus in fresh oysters in production areas and dispatch centres. NRL Ireland provided background information on the national surveys and intervening actions. NRL United Kingdom, former EURL Bivalves, presented a summary on studies towards the relationship between noroviral levels in oysters and shellfish-borne viral outbreaks.

During the meeting also information was shared on SARS-CoV-2 virus (COVID-19) in perspective of foodborne transmission. As documented elsewhere there is no evidence for foodborne transmission, but tests of sewage and water samples for this virus was described as tool to follow the virus spread in the population.

Unfortunately, once more it was concluded there is no financial support of the Commission for harmonisation and standardisation of hepatitis E virus (HEV) in food, despite it is a true foodborne virus.

13.1.2 Participation in working groups

The NRL participated in the 1st meeting of the Next Generation Sequencing (NGS) Working Group for the NRLs for foodborne viruses, September 1st 2020, held as a video conference. Participating NRLs shared their experiences and wishes for the EURL on this item. The main goal for the EURL is harmonisation of typing methods for foodborne viruses. The EURL will focus on amplicon sequencing (metabarcoding). NRL France, Ifremer, gave a presentation on their results of NGS in shellfish samples. Sequencing of viruses in food is much more complex than sequencing from bacterial isolates or clinical specimens, because of the very low viral load in food. The second meeting will be organised proceeding the 4th EURL foodborne viruses meeting in 2021.

13.1.3 Participation in proficiency and comparative tests

The NRL participated in six PTs for detection of norovirus genogroup I (NoV GI), norovirus genogroup II (NoV GII) and hepatitis A virus (HAV) in food or lenticule samples. Of these, three were organised by the EURL, two by Bipea and one by Public Health England (PHE). The NRL also participated in a ring-trial organised by Paul Ehrlich Institute (PEI) and European Centre for Disease Prevention and Control (ECDC) comparing the various typing methods using Sanger sequencing for HEV.

Table 13.1 *Overview of proficiency test and comparative studies NRL Food Borne Viruses*

PT (organisation)	Matrix	Viruses	Assessment
19EFV02 (EURL,SE)	Raspberry 10/19	NoV GI, NoV GII, HAV	100%
PT83 (Bipea, Fr)	Strawberry 1/20	NoV GI, NoV GII, HAV	Partly unsatisfactory [1)]
PT83 (Bipea, Fr)	Strawberry 10/20	NoV GI, NoV GII, HAV	92% [2)]
NHV007 (PHE, UK)	Lenticules 2/20	NoV GI, NoV GII, HAV	100%
20EFV04 (EURL, SE)	Lettuce 10/20	NoV GI, NoV GII, HAV	100%
20EFV05 (EURL, SE)	Oyster 11/20	NoV GI, NoV GII, HAV	100%
HEV (PEI/ECDC, DE)	Serum 1/20	HEV seq (sub) typing	100%

1) According to the organisation, the artificial contamination levels were chosen too low, which explains unsatisfactory outcomes for many of the participants. For future PTs, higher artificial contamination levels will be selected.

2) 11 out of 12 target/samples combination satisfactory, 1/12 not satisfactory.

13.2 Assistance to official laboratories

13.2.1 Quality control

WFSR is both NRL foodborne viruses and the only Official Laboratory for foodborne viruses in food. Data on PT tests have been requested and shared with the NRL foodborne viruses specifically in bivalve molluscs, RIVM.

13.3 Scientific and technical support to the competent authority

In response to discussions on the possibility of a microbiological criterion for norovirus in bivalves in the EU, the ministry of VWS, NRL foodborne viruses in bivalves (RIVM), and NVWA was informed on the detection of norovirus in bivalve molluscs by the Official Laboratory (WFSR) in April 2020. In this videoconference, the NRL foodborne viruses (WFSR) shared and explained data on the prevalence and contamination levels of oysters and mussels collected along the food chain, in the Netherlands, over a long period, 2013-2017. These data have recently been published in the International Journal of Food Microbiology (https://doi.org/10.1016/j.ijfoodmicro.2021.109089).

During the Annual NRL meeting for Dutch NRLs on foodborne pathogens (i.e. Salmonella, Listeria, Staphylococcus Aureus, E. coli, Viruses), October 2020, the Competent Authority (NVWA) and RIVM were updated on the developments in the field of foodborne viruses and activities within the EURL network.

13.4 Contacts with other NRL's

The NRL contacted the EURL in summer 2020 to initiate a study to compare digital RT-PCR with the standardised RT-qPCRs as described in ISO 15216-1:2017 on norovirus detection and quantification in oyster PT samples. The initiative was accepted by the EURL and 8 to 10 NRLs were willing to participate in the study. WFSR was appointed project leader to further elaborate this initiative. Due to COVID-19 the comparison study was postponed to 2021.

14 National Reference Laboratory moisture in poultry meat

Coordinator: Erika Siletti

14.1 Activities within the EURL-NRL network

In 2020, the COVID-19 pandemic and subsequent restrictions to travel have affected the activities of the EURL-NRL expert group 'Water content in poultry meat'. Normally this NRL takes part in two meetings organised every year together with the other European NRLs and the responsible representatives from Brussels. The first meeting is usually organised in Brussels, while the second meeting (2-days meeting) is usually hosted by one of the EU-members.

14.1.1 Participation in EURL-NRL workshops

Expert meeting in Brussels.
The first meeting of the Expert group 'Water in Poultry meat' for the year 2020 was supposed to be held on 23rd March 2020. Unfortunately, this was cancelled about one week before the actual meeting and no other meetings have been scheduled in 2020 to replace this one.

Expert meeting hosted by one of the EU-member countries.
The meeting was planned for October in Slovakia (no date was set). According to a communication in November 2020, the organisation decided to postpone it, due to COVID-19, to 2021. No other communication has reached the NRL and no information is at this stage available about a possible future meeting.

14.1.2 Participation in proficiency and comparative tests

No proficiency test (PT) among NRLs was organised by EURL in 2020. Normally these PTs are organised following detailed discussions with the other NRLs. Unfortunately, due to the cancellation of the meetings, such discussions did not take place. However, EURL would like to start again discussing the topic of a possible future PT in 2021.

14.1.3 Quality control

The Dutch NRL has organised two quality control rounds (April 2020 and September 2020) in order to ensure that the official laboratory (OL) and NRL obtain statistically similar results for moisture and protein analysis in poultry meat. The quality controls comprised an interlaboratory check of the moisture and protein analysis conducted on homogenised and not homogenised samples of chicken fillet meat and chicken legs. The results of both rounds lead to the observation that moisture and protein analysis were acceptably reproducible on an interlaboratory basis. However, for the chicken legs (bone containing samples) the limits of interlaboratory reproducibility were in some cases exceeded in both quality control rounds.

14.1.4 Advice

Common causes for reproducibility issues on poultry cuts which contain bones are unavoidable variation in sample material and differences in homogenisation practices. Therefore, it will be advisable to start investigating how the differences between the homogenisation practices between OL and NRL are truly affecting the results of moisture and protein analysis in poultry meat.

14.2 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 where the NRL experts were consulted.

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