National Reference Laboratories Wageningen Food Safety Research

Annual report 2022

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Wageningen, November 2023

WFSR Report 2023.014

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P.O. Box 230, 6700 AE Wageningen, The Netherlands, T +31 (0)317 48 02 56, E info.wfsr@wur.nl, www.wur.eu/food-safety-research. WFSR is part of Wageningen University & Research.

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Preface

Wageningen Food Safety Research (WFSR) has been entrusted with several reference tasks concerning the safety and quality of food and feed. These reference tasks are carried out as part of WFSR's role as a National Reference Laboratory (NRL) in various fields. As part of official national controls, NRLs and Official Laboratories (OLs) conduct analyses on food and feed. NRLs act as the bridge between European Union Reference Laboratories (EURLs) and OLs and provide expertise to the OLs, Ministries, and competent authority.

In this annual report, WFSR reports on the execution of its NRL tasks in 2022.

Summary

National Reference Laboratories (NRLs) are part of the system responsible for controlling and enforcing EU food and feed legislation. Wageningen Food Safety Research (WFSR) has been designated as NRL for thirteen areas of competence including milk and milk products, marine biotoxins, animal proteins in feedingstuffs, certain substances and residues thereof[1], additives for use in animal nutrition (feed additives), genetically modified organisms 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. The tasks of WFSR's NRLs depend on their respective fields of research. This annual report summarises all the activities carried out by WFSR's NRLs in 2022.

Staying updated on the latest developments is a crucial aspect of NRL's responsibilities. The European Union Reference Laboratories (EURLs) arrange one or two workshops annually to facilitate this objective. Attendance at these workshops is mandatory for EURL-NRL members. In 2022, WFSR's NRLs attended all the workshops. They actively participated in the EURL working groups to enhance their analytical methods.

To assess the analytical capabilities of NRLs, EURLs conduct proficiency tests. Since the scope of these tests is sometimes limited, WFSR's NRLs also participated in proficiency tests organised by other relevant organisations. The majority of the proficiency tests yielded satisfactory results, with only a few instances of 'questionable' or 'unsatisfactorily' outcomes. These were addressed through follow-up actions.

To ensure the performance of Official Laboratories (OLs), their results in proficiency tests organised by WFSR's NRLs were checked, or assurance samples were provided. Additionally, technical support related to their analyses was provided for some OLs.

Furthermore, the scientific and technical backing offered to the competent authority is explained. In some cases, contact with other NRLs is also discussed.

[1] Article 150 of Regulation (EU) 2017/625: Competent authorities shall continue to perform the official controls necessary to detect the presence of the substances and groups of residues listed in Annex I to Directive 96/23/EC, in accordance with Annexes II, III and IV to that Directive, instead of the corresponding provisions of this Regulation, until 14 December 2022 or an earlier date to be determined in the delegated act adopted in accordance with paragraph 3 of this Article.

1 Introduction

Coordinator: Yvette Hoffmans

Within the European Union, an extensive framework of food law is established with the main aim of ensuring a high level of protection of human health and life and achieving the free movement of food and feed. Whereas food and feed businesses have to comply with the requirements of food law, the competent authorities of Member States are to enforce food law and monitor compliance with it. It is the task and the competence of the competent authorities to verify that the relevant requirements are fulfilled by food and feed business operators at all stages of food and feed production. This is done by executing official controls, and requirements thereof are prescribed in European Union and national rules. Official samples for analytical analyses are taken to execute the official control. The multi-annual national control plans (MANCPs) set up by the competent authorities cover a large part of the samples for official controls. Requirements have been set for laboratories, sampling, and analytical methods to ensure the uniformity of analytical results for the official controls. For this purpose, European Union Reference Laboratories (EURLs) contribute to improving and harmonising analysis methods and support National Reference Laboratories (NRLs). Every Member State is obliged to designate at least one NRL for each EURL. NRLs are expected to stay up-to-date with scientific advances within their field and are tasked with supporting 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) (Staatscourant Nr. 35329, https://zoek.officielebekendmakingen.nl/stcrt-2019-35329.pdf).

These subjects are:
- Animal proteins in feedingstuffs
- Additives for use in animal nutrition
- Foodborne viruses
- Genetically modified organisms (GMOs)
- Halogenated persistent organic pollutants (POPs) in feed and food
- Marine biotoxins
- Metals and nitrogenous compounds in feed and food
- Mycotoxins and plant toxins in food and feed
- Processing contaminants
- Residues of pesticides
- Residues of veterinary medicines and contaminants in food of animal origin
- Milk and milk products*
- Moisture in poultry meat**

*: For ‘Milk and milk products’, the EURL was deemed not required anymore as of 1 January 2018. However, the Dutch ministries decided to keep an NRL for ‘Milk and milk products’.
**: The NRL for moisture in poultry meat is appointed under the Single Market Regulation.

This report aims to provide an overview of activities performed by the NRLs of WFSR in 2022.

1.1 EU-Legislation

The requirements regarding official controls performed to ensure compliance with feed and food law, as well as animal health and animal welfare criteria, are laid down in the Official Control Regulation (OCR), Regulation (EU) 2017/625. This regulation mandates that the Member States uniformly monitor and verify that the relevant requirements are fulfilled at all stages of food production, including processing and distribution. The OCR describes the requirements for EURLs, NRLs and OLs as well.

In addition to this regulation, more specific legislation applies to certain parts of the production chain or particular subjects. For instance, Regulation (EC) No 543/2008 lays down additional provisions for official controls on the water content of poultry. Further, specific provisions for the official control of residues of veterinary medicines and banned substances in the production of animals for food production are laid down in Regulation (EU) 2019/6.

1.1.1 Competent authorities

According to the OCR, the official controls are under the responsibility of the competent authority assigned by each Member State. Within the Netherlands, the Food and Consumer Product Safety Authority (NVWA) is assigned as competent authority by the Ministry of LNV. In its turn, the NVWA is responsible for assigning OLs to execute the analysis of samples for the purpose of official controls. Furthermore, the NVWA is responsible for making the multi-annual national control plan, which includes sample analysis in the different food and feed supply chains. Besides that, the enforcement of food law falls under the responsibility of the NVWA, which also means that the NVWA has to act in case of incompliant analysis results.

1.1.2 European Union Reference Laboratories (EURLs)

EURLs are designated by the European Commission (EC). Laboratories have been invited to become EURL via a tendering procedure. Table 1.1 shows the EURLs relevant to the NRLs of WFSR (https://food.ec.europa.eu/horizontal-topics/european-union-reference-laboratories_en).

Table 1.1 *List of EURLs relevant for NRLs WFSR.*

Subject/substances/products	***EURL***
Additives for use in animal nutrition	The Joint Research Centre of the European Commission Belgium
Animal proteins in feedingstuffs	Centre wallon de recherches agronomiques (CRA-W) Belgium
Foodborne viruses	Livsmedelsverket Sweden
Genetically modified organisms (GMOs)	The Joint Research Centre of the European Commission Italy
Halogenated persistent organic pollutants (POPs) in feed and food	Chemisches und Veterinäruntersuchungsamt (CVUA) Germany
Marine biotoxins	Agencia Española de Seguridad Alimentaria (AESA) Spain
Metals and nitrogenous compounds in feed and food	National Food Institute, Technical University of Denmark (DTU) Denmark
Mycotoxins and plant toxins in feed and food	Wageningen Food Safety Research (WFSR) The Netherlands
Processing contaminants	National Food Institute, Technical University of Denmark (DTU) Denmark
Residues of pesticides:	
Cereals and feedingstuffs	Fødevareinstituttet Danmarks Tekniske Universitet Denmark
Food of animal origin and commodities with high-fat content	Chemisches und Veterinäruntersuchungsamt (CVUA) Germany
Fruits and vegetables, including commodities with high water and high acid content	Laboratorio Agrario de la Generalitat Valenciana (LAGV) Spain Grupo de Residuos de Plaguicidas de la Universidad de Almería (PRRG) Spain
Single residue methods	Chemisches und Veterinäruntersuchungsamt (CVUA) Germany

Subject/substances/products	*EURL*
Residues of veterinary medicines and contaminants in food of animal origin:	
Mentioned in Annex I, Group A 1 2 3 4, B2d, B3d Directive 96/23/EC	Wageningen Food Safety Research (WFSR) Wageningen The Netherlands
Annex I, Group B1, B3e Directive 96/23/EC and carbadox and olaquindox	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES) France
Annex I, Group A5, B2 a b e Directive 96/23/EC	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) Germany
Other:	
Milk and milk products	No longer required in the EU as of 1 January 2018, but the Dutch ministries decided to keep an NRL for 'Milk and milk products'.
Water content poultry meat	Board of Experts: JRC (IRMM), DG AGRI and three NRLs*

* Note: The board of experts is referred to 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 as 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 the Member States 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 for experts from third countries;
f. providing scientific and technical assistance to the Commission within the scope of its 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 the 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, and animal proteins in feedingstuffs, milk and poultry meat. Work plans to describe the tasks for 2022 were drawn up in 2021. In addition, budgets for staff costs and costs for facility and equipment were prepared. The 2022 work plans were positively reviewed by the Client Consultation Board (consisting of employees of the NVWA, the Ministry of LNV and the Ministry of VWS) and approved by the Ministry of LNV. The work plans are based on the NRL tasks 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, participate in training courses and 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 view of harmonising and improving the methods of laboratory analysis, test or diagnosis 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 competent authorities of the results of such tests and follow-up;
d. ensure the dissemination to the competent authorities and OLs of information that the EURL supplies;
e. provide within the scope of their mission scientific and technical assistance to the competent authorities 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 feed additives (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 cited as the NRL in: Decision 98/536/EC (residues of veterinary medicine and hormones (Directive 96/23/EC) (both no longer in force)), 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

According to Article 37 of Regulation (EU) 2017/625, the competent authorities are to designate 'official laboratories' (OLs) authorised to carry out the analyses on samples taken within the context of official controls. competent authorities may only designate laboratories that operate and have been assessed and accredited in accordance with the European standard EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. These accreditation requirements also apply to NRLs.

1.1.5 Methods of analysis

The analysis methods 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 1.1 *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/11312/2021	• 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)
Regulation (EC) 2021/808	• Residues of veterinary drugs and hormones
Regulation (EC) 641/2004	• GMO
Regulation (EC) 619/2011	• GMOs in feed (for GMOs with pending authorisation or expired authorisation)
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 1 January 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 2022. However, several member states, including the Netherlands, have expressed the intention to keep their NRL MMP active. NRLs of these states share the belief 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 meetings of a voluntary cooperative network of NRLs MMP on 19-20 September 2022 in Rapotin, Czech Republic. During the workshop, the 10 NRLs shared the general structure of their NRL role in their country and looked for ways to continue cooperation. One suggestion is to create a newsletter with updates on methodology in the field, also issued to laboratories that do not have a formal NRL task anymore, but also to several Official Laboratories in Europe to create more common ground. A (bi)annual meeting was also suggested. The Czech host also organised short excursions to an A2-milk dairy farmer and a dairy processor, which was also valuable for the participants to see the necessity of their laboratory work in practice.

2.1.2 Participation in proficiency and comparative tests

The NRL participated in several international proficiency tests to keep the quality of its methods up to date. The overview and results are presented in Table 2.1.

Table 2.1 *Overview of participation and results of NRL MMP performance trials in 2022.*

Organiser				**Performance[1]**			
Country (code)	**Date**	**Matrix**	**Analyte[2]**	**z-scores**	**S**	**Q**	**U**
ALP, CH (21A)	Jan	Stabilised milk	SCC	-0.2 – 1.6	*		
ALP, CH (21B)	May	Stabilised milk	SCC	-0.6 – 0.8	*		
ALP, CH (22C)	Sept	Stabilised milk	SCC	-0.8 - -0.1	4		
Cecalait, FR	March	Milk	SCC	-3.9 – 1.0	3	4[a]	3[a]
Cecalait, FR	June	Milk	SCC	-3.9 – 2.0	8	1[a]	1[a]
Cecalait, FR	Sept	Milk	SCC	-7.9 – -2.0		2[a]	8[a]
Cecalait, FR	Dec	Milk	SCC	-8.0 – -0.5	2		8[a]
LGC, UK	Jan	Stabilised milk	ALP	-3.8 – 0.8	1		1[b]
LGC, UK	May	Stabilised milk	ALP	-0.6 – 0.4	2		
LGC, UK	Aug	Stabilised milk	ALP	0.3 – 0.5	2		
LGC, UK	Nov	Stabilised milk	ALP	-0.8 – 0.0	2		
Cecalait, FR	Jan	Milk	TF	-3.5 – 0.8	9		1[c]
Cecalait, FR	April	Milk	TF	-2.9[d] – 5.0[d]	-[d]	-[d]	-[d]
Cecalait, FR	Dec	Milk	TF	-0.8 – 9.6	9		1[c]

[1)] Performance: z-score range and number of (S)atisfactory, (Q)uestionable and (U)nsatisfactory results.

[2)] SCC= somatic cell count; ALP= alkaline phosphatase; TF= total flora.

*) Evaluation is not available at the time of writing. An inquiry has been sent to the organisers in January 2023.

[a-d)] Refer to explanation in the text.

Most results in the ring trials were satisfactory, but there were exceptions. For the Cecalait SCC trials ([a]), results from this year were frequently below the assigned values. These results follow the generally good scores in 2021 and poor scores in the years before 2021. In the years before 2021, poor performance was explained because of the inclusion of participants with routine instruments, which have low variance and a bias against the reference method. Since the JRC ERM-BD001 SC reference materials became available, WFSR's reference methods proved fine and no adjustments were needed, whereas internationally many routine labs were required to make adjustments. This also led to a shift in assigned values in the Cecalait ring trial. It remains to be investigated whether Cecalait's assigned value is too high or whether WFSR's method is of insufficient control. Performance on the ERM and in the Swiss ALP ring trial on SCC suggests WFSR is performing well on SCC count, but more checks and international consultation are needed to conclude the matter.

One unsatisfactory result for LGC's ALP trial in January[(b)] was due to a typing error during reporting. The true analytical result would have yielded a z-score of 0.8, which is considered satisfactory.

Cecalait's TF rounds of January and December [(c)] both had one deviating sample, without any traceable problems for these particular samples. The other 9 samples in this round were very close to the assigned values, and no further action was deemed necessary.

WFSR's result series for the Cecalait TF round of April [(d)] is considered invalid. The samples received in this round were stored inadequately and too long before analyses; proper storage obviously matters for microbiological assays. Due to problems in internal communication, this was noticed only after the samples had been analysed. Since the results were available, they were still submitted, but as expected, they proved mainly unsatisfactory.

2.2 Assistance to official laboratories

2.2.1 Quality control

In 2022, the NRL assisted 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 SCC method. The NRL re-analysed two series of routine cheese samples from the OL to compare the alkaline phosphatase levels obtained by NRL and OL. For total flora, it was arranged that the NRL and OL participated in the same series of Cecalait PTs (paragraph above). Thus the results on the same material could be used for interlaboratory comparison.

2.2.2 Advice

The NRL advised the competent authority (COKZ (Controle Orgaan KwaliteitsZaken)) and the official laboratory on the process of implementing the reference material on somatic cells (JRC ERM-BD001) into routine analyses. In 2022, the NRL had meetings with the Netherlands Food and Consumer Product Safety Authority (NVWA) on the need for official control of official routine analyses of 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. The NRL applied for accreditation for a few extra matrix/product combinations as part of this new work. Discussions on the mode for NRL-quality control of the new tasks were undertaken. Still, some decisions on supporting the competent authority in fulfilling their supervisory task towards the official laboratories remain to be taken further in 2023.

3 National Reference Laboratory Marine biotoxins

Coordinator: Mirjam Klijnstra

3.1 Activities within the EURL-NRL network

3.1.1 Participation in EURL-NRL workshops

The EURL changed staff on January 1st, 2022. An online start meeting was organised in March in which the new EURL staff was introduced, and the work programme for 2022 was presented.

On 20 and 21 October 2022, an EURL workshop was organised in Vigo, Spain. During the workshop, the following was discussed:

- Proficiency testing schemes, organisation, evaluation and results of the proficiency tests (PTs) for domoic acid (ASP), lipophilic toxins and paralytic shellfish toxins (PSTs) in bivalve molluscs;
- All NRLs presented their institute, their monitoring programme and, if applicable, some of their research results;
- A new working group (WG) on toxic phytoplankton detection was discussed. Given the current development and availability of molecular methods for the detection of toxic phytoplankton that could be readily used for monitoring and early warning purposes, a new WG with experts in this field will be established and coordinated by the EURLMB (EU Reference Laboratory for Marine Biotoxins). The objectives of this WG are to elaborate a report by the end of 2023 with the state of the art of molecular techniques for the detection of toxic phytoplankton, plus technical guidelines and protocols to facilitate their application in monitoring systems;
- A call for experts was issued for a new WG on the classification and monitoring of production areas for bivalve molluscs;
- The EURL gave a presentation on "Samples and preliminary results for the analysis of ASP, PSTs and lipophilic toxins in non-traditional vectors";
- Issues proposed by the EURL/NRLs were discussed. Issues brought in by the Dutch NRL were the wish to change the legislation towards method performance criteria instead of prescribed methods and the use of recovery in the EN 14527:2017 method for the analysis of PSTs.

3.1.2 Participation in a working group

On October 19th 2022, a working group on emerging toxins was organised prior to the EURL workshop in Vigo, Spain. There were presentations from invited experts, and the participating NRLs presented their work on emerging toxins. The conclusions were:

- The group expresses that high priority should be given to harmonising the cell-based assay method for ciguatoxins. EU NRLs express the need for having common materials for intercomparison. EURLMB is to schedule a specific WG in 2023 for this SOP elaboration and to ask the group for the possibility of sharing materials for comparisons;
- The participants of the WG on emerging toxins recognised the concern about the presence of tetrodotoxin levels above the maximum EFSA recommended levels in certain EU gastropods and bivalve molluscs because these toxins are not regulated in the EU;
- Participants want the EU commission to request EFSA to evaluate the risk for human health associated with cyanotoxins from fresh, brackish and marine waters in marine bivalve molluscs;
- WG Participants propose to schedule an interlaboratory trial for cyclic imines in 2023.

3.1.3 Participation in proficiency and comparative tests

The NRL participated in the EURL PTs on ASP, PSTs and lipophilic marine toxins in shellfish and in PTs on PST and TTX in shellfish organised by QUASIMEME. The results are presented in Table 3.1.

Table 3.1 *PT results Dutch NRL marine biotoxins in bivalve molluscs.*

PT	Analytes	z-scores
EURLMB-22-A	Domoic acid	2.7 and 1.6
EURLMB-22-L	Lipophilic toxins	Between -1.6 and 0.7
EURLMB-22-P	Paralytic shellfish toxins	Between -1.1 and 1.4
QUASIMEME BT12	Paralytic shellfish toxins	2021 round 2: Between 1.3 and 3.6 2021 round 2*: Between -0.9 and 0.3 2022 round 1: Between -2.5 and 5.5 2022 round 1*: Between -2.5 and 5.5
QUASIMEME DE16	Tetrodotoxins	-0.1 and -0.3

* results uncorrected for recovery.

For ASP, two PT samples were analysed with the EU reference method using HPLC-UV. The result of one sample was satisfactory (z-score < |2|), and the other sample gave an unsatisfactory result (z-score > |2|). The existing method was not used properly. To prevent this error in the future, the method was further optimised in 2022 and will be validated in 2023.

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

Two EURL and six QUASIMEME PT samples for PSTs were analysed using the HPLC-FLD method (OMA 2005.06). In the EURL PT, for the individual toxins, all 7 reported values were satisfactory (z-score < |2|). In the QUASIMEME PT, 10 of the 25 reported values yielded unsatisfactory results. Eight unsatisfactory results can be linked to the recovery correction applied by WFSR. Most of the laboratories participating in the PT do not apply a true recovery correction within their analysis. The Dutch NRL believes that a recovery correction should be applied, and it has therefore submitted the recovery-corrected results as they would have been reported in official analyses. However, to evaluate the performance of the Dutch NRL compared to the other laboratories, the Dutch NRL also calculated the results without recovery correction. All but two results would have been satisfactory without applying the recovery correction. The latter unsatisfactory results were due to the LOQ of the method and an error while processing the results. For the latter, the results were recalculated and were then satisfactory.

WFSR participated in a development exercise for TTX organised by QUASIMEME. Both submitted results were satisfactory (z-score < |2|).

3.2 Assistance to official laboratories

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

3.3 Scientific and technical support to the competent authority

The NRL prepared a publication on the presence of cyanobacterial toxins in Dutch surface waters. These data were gathered for NVWA-BuRO in 2020 and 2021 and were, on request of NVWA-BuRO, written down in a draft scientific article, which will be submitted for peer review in 2023.

During the annual NRL meeting, the competent authority (NVWA), the Ministry of Health, Welfare and Sport, the National Institute for Public Health and the Environment (RIVM) and Wageningen Marine Research (WMR) were updated on the developments in the field of marine and cyanobacterial toxins. These developments included cell-based assay screening of samples from the sanitary shellfish monitoring program, a survey on the cyanobacterial toxin cylindrospermopsin in shellfish, the marine biotoxin guide, which was developed under the coordination of the EURL, method development on ciguatera toxins in fish, a survey on palytoxins in shellfish and method development on the detection of bound microcystins. Also, data on the presence of cyanobacterial toxins in Dutch surface waters were presented and the possibilities of detecting domoic acid using Immuno-enriched microspheres magnetic blade spray mass spectrometry. The work plans for 2022 were presented.

3.4 Contacts with other NRLs

WFSR is also the NRL for Northern Ireland. WFSR started as NRL for Northern Ireland at the end of 2021. The Dutch and Northern Irish NRL tasks will be carried out independently unless in cases where it is more efficient to combine tasks, for example when participating in proficiency tests. If tasks of both NRLs are combined, WFSR will ensure this does not lead to conflicts of interest.

Furthermore, there was contact with the NRLs of Germany, France, Belgium and Sweden to exchange materials or knowledge on several toxin groups. Materials containing CTX and TTX were shared, and how to set up a validation for certain methods or toxin groups was also discussed.

4 National Reference Laboratory Animal proteins in feedingstuffs

Coordinator: Guus van der Borg

4.1 Activities within the EURL-NRL

4.1.1 Participation in EURL-NRL workshops

The annual meeting 2022 of the EURL/NRL network was organised in Leuven over two sessions on the 26th and 27th of April. WFSR participated actively in both sessions.

4.1.2 Participation in Working groups

Working groups on microscopy and Polymerase Chain Reaction (PCR) are currently not active.

4.1.3 Participation in proficiency and comparative tests

The EURL-AP organised a combined microscopy-PCR Proficiency Test (Fumière et al., 2023) during the winter of 2022. The test consisted of seven samples. The preliminary feedback revealed that one sample was a blank (a clean poultry feed), and the other six samples were contaminated with materials of terrestrial animal origin. None of the samples was contaminated with materials of fish origin. Four samples were spiked with Processed Animal Proteins (PAPs), whilst the remaining two were spiked with milk powder or feather meal. The Dutch NRL provided unsatisfactory results according to the EURL; however, these results were formally disputed. The EURL did not change the evaluation after the dispute.

The results of the Dutch NRL were evaluated as unsatisfactory due to the misidentification of two separate samples in the microscopy part of the PT. In one sample the Dutch NRL did not identify milk powder, whilst in another sample the Dutch NRL mistakenly identified it as containing material of fish origin. According to the EURL, these two misidentified samples made for an overall PT accuracy of 0.846, which is less than the cut-off of 0.9 and thus considered unsatisfactory. After receiving the preliminary feedback, the Dutch NRL was requested to submit a report on the underperformance and asked to investigate the cause of the underperformance and to formulate an action plan to rectify the discovered issues. This investigation was performed and in the course of the investigation, it was concluded that the report would include a formal request to reconsider the results and to change the result. The main reasons being that:

1. The monitoring duties do not require the identification of milk powder in samples.
2. Identifying milk powder in samples may be impossible if the method is performed according to the Regulation that describes the method.
3. The method has never been validated for identification of milk powder.

The first reason is, admittedly, still disputed by the EURL. However, all relevant legislation (Regulation (EC) No 999/2001, Regulation (EC) No 152/2009, and Regulation (EC) No 142/2011) and the official SOP published by the EURL explicitly exclude milk and milk products from the scope of the monitoring duties.

The second reason is a result of Regulation (EC) No 152/2009, which prescribes the method to be used, explicitly listing 2.5% w/w NaOH solution as one of the allowed mounting media for microscopy slides. However, as this is an aqueous solution, milk powder will dissolve in this mounting medium, making it impossible to detect milk powder using visual methods.

The third reason means exactly what it implies. All attempts to validate the method for the use of identifying milk powder have shown unsatisfactory results. All the tests that have been performed to quantify the capability of the method to identify milk powder have shown that only 30-59% of participating laboratories

were found capable of identifying milk powder (including the current EURL-PT, where 50% of the participants did identify the milk powder), with the 59% trial only being so high due to the samples being labelled as containing milk powder. This is not sufficient to validate a method as viable for identifying milk powder.

Due to these reasons, the EURL was requested to label the milk powder sample as a 'challenger' sample that did not count towards the accuracy of a participating laboratory, as three laboratories were given an 'unsatisfactory' result by not being able to identify milk powder. Having received these explanations by the Dutch NRL, the EURL did not change the final report, nor commented on the request to relabel the milk powder sample. The Dutch NRL is currently in discussion with the EURL on the necessity of identifying milk powder, and other similar feed components, in samples.

To comment on the second misidentified sample, in a sample containing mineral premix + 0.5% tricalcium phosphate, the Dutch NRL mistakenly identified the sample as containing particles of fish origin. After investigating the issue, it was found that the sample contained an unusually high amount of tooth particles. Tooth particles are practically indistinguishable from particles of fish origin. In the vast majority of cases, however, there are not enough tooth fragments in a sample to lead to a positive identification. As such, this sample was considered a statistical outlier.

4.2 Assistance to official laboratories

The Netherlands does not maintain a national network of official control laboratories. 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 following issues and subjects have been communicated with the Dutch Ministry of Agriculture, Nature and Food Quality and the Dutch competent authority: WFSR, as the Dutch NRL, has published the Quality Guidance for Visual Research in cooperation with a panel of European specialists (van Raamsdonk et al., 2022), and is working on getting this guideline formalised as a CEN standard.

The IAG section Feed Microscopy animal proteins Proficiency Test was organised by WFSR in 2022.
Fifty participants from 20 different countries participated. Of these 20 countries, 14 were member states of the European Union. The PT successfully concluded with a published report (WFSR report 2022.010).

4.4 Contacts with other NRLs

WFSR is a member of the IAG section for Feed Microscopy. The EURL AP and most NRLs are members of this section. During the IAG annual meeting in June every year and, if necessary, during other meetings, exchange of viewpoints and other 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 frequently discussed in the meetings and complements the information of the EURL/NRL AP network. The IAG PT for animal proteins was again organised in 2022. The meeting was held at Masterlab Nutreco in Boxmeer, The Netherlands, from the 7th-9th of June.

4.5 References

Raamsdonk, L.W.D. van, G. Frick, I. Ujcic Vrhovnik, M. Zadravec, J. Zegers, R. Krull-Wöhrmann, R. Weiss, G. van der Borg, 2022. Quality assurance and control of visual methods for feed and food inspection. Part 1 – Theory and principles, Part 2 - Validation. Report 2022.006. WFSR, Wageningen, pp. 70.

Veys, P., Fumière, O., Marien, A., 2023. Combined microscopy-PCR EURL-AP Proficiency Test 2022. Final version. European Union Reference Laboratory for Animal Proteins in feedingstuffs, Walloon Agricultural Research Centre, Gembloux, Belgium.

5 National Reference Laboratory Directive 96/23/EC

Residues of veterinary medicines and contaminants in food of animal origin: Group A 1 2 3 4, B2d, B3d, Directive 96/23/EC Annex I

Coordinator: Johan Lasaroms

5.1 Activities within the EURL-NRL network

5.1.1 Participation in EURL-NRL workshops

The Dutch NRL participated in the EURL-Workshop organised by BVL-Berlin, which was, due to the ongoing COVID-19 pandemic and the restrictions on travelling abroad, held online on the 5th and the 6th of May 2022. The Workshop was divided into four sessions: two general informative sessions on an EFSA 2020 report and news from the Commission, one informative session on the calculation of measurement uncertainty and one informative session on the organised proficiency tests (PT) NIIM1021 and some general information related to the EURL Berlin.

Some interesting topics of this workshop were:
- News on the substance groups beta-agonists with an interesting presentation about the revalidation of the analytical method for the detection of 32 different beta-agonists in the matrix urine at low residue levels.
- News on the substance group anthelmintics in which a validated confirmatory method for the detection in the matrix liver originating from bovine and ovine was presented. This method allows the detection of a total of 60 components at low residue levels. A comparison between different types of analytical systems was also presented.
- Results of the proficiency test NIIM1021 (nitroimidazoles in plasma and milk) organised by BVL Berlin were presented. Some conclusions were: 2/3 of the participants have a method for NIIM in milk; very low numbers of false positives; methods applied in the routine analysis are primarily fit for purpose; very good overall results.

The second workshop in which the Dutch NRL participated was the EURL-Workshop organised by ANSES Fougeres. This workshop was held live from the 22nd of June until the 24th of June.
This workshop, 'Control of Antimicrobial Residues in Food from Animal Origin', only comprised a theoretical part, including several presentations.

Some topics of this workshop were:
- Regulatory Issues like Guidance for Screening Methods in line with Regulation 2021/808; News from the Commission.
- EFSA VMPR 2020 report (facts on antimicrobial residues).

Several presentations were given on new or improved methods for detecting residues in various matrices. Some interesting presentations in the field of 'Advances in Multi-Residue Screening HRMS Methods' were:
- Multi-residue method for the screening of antimicrobial VMPRs in eggs by Q-ToF-HRMS.
- Advance in the round 1 collaborative study (21.SR) for the screening by LC-(LR/HR)MS multi-classes of VMPRs in meat and milk extracts.
- Improving QuEChERS extraction-based analyte recoveries: example of 201 veterinary drugs in milk and other solid matrices.

In the field of 'Biological Screening Methods'; some interesting topics were:
- Bio-rapid methods for testing residues of antibiotics in eggs by immunological techniques.
- Evaluation of sensitivities of ELISA testing kits for residues of group A6 prohibited antibiotics at and below their new RPAs according to reg. 2019-1871.

The third EURL-Workshop in which the Dutch NRL participated was organised by and held at WFSR-Wageningen from 7-9 November 2022. This workshop on the control of Growth Promoters Residues in Food from Animal Origin consisted of a theoretical part with several presentations and a practical part with multiple demonstrations in the laboratory.

The scope and aims of the workshop were:
- Bringing together NRLs and discussing topics in the field of growth promotors, tranquilisers and antivirals.
- Reconnecting after two years of online workshops.
- Discussing and giving input into new legislation and Guidance Documents.
- Getting insight into trends in the field.
- Seeing trends in the laboratory.
- Exchanging knowledge between the NRLs.
- Establishing and strengthening long-lasting collaboration between the NRLs.

Some interesting topics and presentations of this workshop were:
- A literature overview of antivirals consisting of an introduction to antivirals and what they are used for, an explanation of the principle of action of antivirals, an overview of viral diseases in food-producing animals, including disease control and outbreaks, and an overview of current existing analytical methods
- RALs research, history and current work.
- Prioritization of substances for a risk-based National Residue Monitoring Plan.
- Scientific presentations of:
  - ecdysteroids method development in-vitro metabolism;
  - GC-HR-MS method for the analysis of steroids in urine.

During the practical part, the following topics were demonstrated:
- Dried Blood Spot analysis.
- Blades Mass Spectrometry.
- Isotope Ratio Mass Spectrometry.
- Broad screening with GC/LC-HRMS.
- Bio effect assays.

Experts from the NRL in the EU Member States and representatives from candidate countries or third countries participated in the abovementioned workshops.

5.1.2 Participation in proficiency and comparative tests

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

Table 5.1 *Overview of proficiency tests.*

Description	Organising institute	z-score	Assessment
Natural Hormones and thyreostats in bovine urine	FAPAS	Between -3.90 and -0.40	Deviate result *)
Quinolones and fluoroquinolones in poultry eggs	FAPAS	Between 0.20 and 1.20	Sufficient
Corticosteroids in bovine meat	FAPAS	Between -1.00 and 0.50	Sufficient
Natural hormones in bovine serum	FAPAS	Between 0.22 and 0.80	Sufficient
Beta-agonists in porcine meat	FAPAS	Between -0.70 and -0.10	Sufficient
Sulphonamides in honey	FAPAS	Between -0.20 and 1.1	Sufficient
Coccidiostats in turkey meat	FAPAS	Between 0.20 and 5.80	Deviate result *)

Description	Organising institute	z-score	Assessment
Nitrofurans and carbadox in porcine liver	FAPAS	Between -0.3 and 0.0	Sufficient
Phenicoles in bovine	FAPAS	Between 0.00 and 2.00	Sufficient
Nitroimidazoles and aminoglycosides in honey	FAPAS	z-score 0.60	Sufficient
Natural hormones and thyreostats in urine	FAPAS	z-score between -0.30 and 0.10 Concordance	Sufficient
Chloramphenicol and beta-agonists in water	Progetto	Between -0.74 and 3.87	Deviate result *)
Sulphonamides in egg	Progetto	Between -0.59 and 0.31	Sufficient
Nitroimidazoles in bovine meat	Progetto	Between -0.28 and 0.17	Sufficient
(Fluoro)quinolones in honey	Progetto	Between -0.08 and 1.17	Sufficient
Tranquillisers Sedatives in porcine kidney	EURL-WFSR	z-scores between -0.85 and 0.77 Concordance 0%	Deviate result *)
(Pro)hormones and stimulants in supplements	WFSR	Concordance 100%	Sufficient
Dyes in trout	EURL-ANSES	Between -3.2 and 0.00	Deviate result *)
Avermectines, dyes and antibiotics in salmon	WFSR	z-scores between -3.09 and 0.22 Concordance 100%	Deviate result *)
Antibiotics in meat	WFSR	Between -0.81 and 0.81	Sufficient
Steroids in urine	EURL -WFSR	z-scores between -1.12 and 0.64 Concordance 100%	Sufficient

Cumulative results are presented in Table 5.2 below.

Table 5.2 *Cumulative results PTs.*

Total z-scores	113	% of total
neg z-score	49	4%
pos z-score	64	57%
z-score <=2 en >=-2	103	91%
z-score <-2	5	4.9%
z-score > 2	5	4.9%
z-score <-3	4	3.9%
z-score >3	3	2.9%

*) Corrective actions are taken in response to the deviating results:

Natural Hormones and thyreostats in bovine urine; the z-score of the compounds tapazole and thiouracil were below -3.
Corrective action taken: several calculations were checked and found to be in order. The sample was re-analysed with an updated method. It was also checked at the organisation of the proficiency test that the sample was stabilised before shipping, which was confirmed. Finally, it was found that proper dilution steps were not performed during the sample preparations. Taking this into account, the assigned value of tapazole and thiouracil was found to be correct.

Coccidiostats in turkey meat; the z-score of diclazuril was above 3.
Corrective action taken: the PT sample was re-analysed using a new and freshly prepared standard solution. With the outcome of the re-analysis, the z-score of diclazuril was set to 2.4, which is still too high but much better than in the first analysis. This time, it was also concluded that no check was performed on the standard solution used for spiking the control samples. This has been discussed with those responsible, and, if possible, this PT sample will be re-analysed. No further corrective action was needed.
Chloramphenicol and beta-agonists in water; the z-score of clenbuterol was above 3.
Corrective action taken: the calculations were checked again, and it was concluded that a mistake was made

when entering the concentrations in the data analysis of the multi-level standard addition.
A sufficient z-score was obtained when the correct concentrations were used.

Sedatives in porcine kidney; azaperone was not detected in the PT sample and therefore reported as not detected. The use of an incorrect method caused this.
Corrective action taken: a procedure has been started to correct the method in order to reduce the number of false-negative results. The current SOP will be modified and validated for this purpose.

Dyes in trout and Salmon; this was a combined PT for several compound classes. Due to the prioritisation of the activities in the laboratory, the PT samples for the dyes were performed ten months after receiving the PT samples. (Leuco)-malachite green is known to be unstable, especially when several freeze/thaw cycles have been applied. In this case, the quantitative result of the compound (leuco)malachite green differs from the assigned value.
Corrective action: It was concluded that due to the instability of the compound, a deviating result was understandable.

5.2 Assistance to official laboratories

5.2.1 Quality laboratories

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

The NRL task (supervising 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.
In 2022 the control program included 63 analyte/matrix combinations, and 55 different analytical methods were tested. In total, 288 quality control samples were analysed, which resulted in 594 results. Twenty-eight results give a deviate result (in total 4.7%), slightly more than in 2021. The overall conclusion is that the assurance of the chemical analyses in 2022 went well and that the outcome of the results is in line with previous years. Appropriate actions were taken for the deviating results.

5.2.2 Advice

The Q3 meeting (consisting of the NRL, OL and another Dutch laboratory) was held twice in 2022, on the 1st of February and on the 22nd of November.

In 2022 the NRL participated in four meetings for the National Plan Residue Control working group; these meetings were held on the 7th of February, the 16th of May, the 10th of October and the 19th of December.

In 2022 the NRL finalised a factsheet on salicylic acid, which was also sent to the competent authority (NVWA).

The NRL has sent a letter to the competent authority (NVWA) on the shelf life of biological materials and the maximum related period within which a re-analysis or a contra-expertise analysis should be performed. This is in response to new insights obtained from stability studies of quality control materials and re-analysis of non-compliant materials.

6 National Reference Laboratory Additives for use in animal nutrition and national evaluation of dossiers / advice

Coordinator: Anthony Verschoor

6.1 Activities within the EURL – NRL network

6.1.1 Participation in EURL-NRL workshops

Wageningen Food Safety Research (WFSR) is the Dutch NRL both for Feed Additives (FA) Authorisation (Reg. (EC) No 1831/2003) as well as for Feed Additives Control (Reg. (EC) No 882/2004 and its successor Reg. (EU) 2017/625). The Dutch NRL participated in the 22nd and 11th Annual Workshops (authorisation & control activities, respectively) of the European Union Reference Laboratory for Feed Additives (EURL-FA) and the consortia of National Reference Laboratories (NRLs). These were organised as a joint webinar, held on November 23-24, 2022.

On the first day, after the introduction of the EURL FA, an overview was given on the EURL-FA Control activities over 2022 by the EURL (Joint Research Centre, JRC-Geel, Belgium). Similar to previous years, the Dutch NRL was among the top 6 NRLs (out of 24 NRLs) that reviewed a large part of the reports received (Dutch NRL: 14/34), even though the Dutch NRL never reviews reports on enzymes or bacteria (18/34). After that, presentations were given on the determination of selenomethionine, on the activities of the EFSA FEEDAP panel on dossiers in 2022, on methods for determination of vitamin D3, on the determination of antibiotics in feed, on the implications of the Transparency Regulation (EU) 2019/1381 by EFSA, and DG SANTE gave an update on the revisions of Regulation (EU) 152/2009 & Directive 2002/32/EC.

On the second day, a presentation was given on the activities of the EURL-FA on authorisation, followed by presentations on methods for the identification of microorganisms, chromium propionate & vitamin B12 and an update on the VDLUFA working group organic feed additives. After that, a discussion was held on topics proposed by the NRLs (including on determination of vitamins and coccidiostats proposed by the Dutch NRL, see also 6.1.4.). Finally, a discussion was held on possible activities of the EURL in 2023, including topics for workshops (antibiotics, p-phenetidine, coccidiostats) and topics for a proficiency test.

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

In 2022, the Dutch NRL commented on 14 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 (emulsifiers), sensory additives (flavouring compounds), zootechnical additives (physiological condition stabilisers, digestibility enhancers, other zootechnical additives) and nutritional additives (amino acids, their salts and analogues, trace elements and vitamins, provitamins and chemically well-defined substances having a similar effect).

6.1.3 Participation in proficiency tests

In 2022, the Dutch NRL-FA participated in one proficiency test organised by the EURL in July 2022 on vitamin A in rabbit feed (1 sample). The results are not available yet.

6.1.4 Contact on methodological issues

In late 2022, assistance was requested from the EURL on two methodological issues: quantification problems with coccidiostats at additive level and repeatability of levels of vitamins A, D and E in animal feed using the CEN 17547 method. Further investigation of these issues is scheduled for 2023.

6.2 Scientific and technical support to the competent authority

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

In The Netherlands, parties may carry out scientific experiments involving the mixing of non-authorized additives in feed. Temporary use exemptions are granted by the Ministry of Agriculture, Nature and Food Quality (LNV), and requests are coordinated by the Dutch Medicines Evaluation Board, Veterinary Medicinal Products Unit, which sends the requests to various agencies to evaluate possible safety issues. WFSR, the Dutch NRL-FA, evaluates the mixing and processing in feed and the possible risks of cross-contamination to other feeds at the feed producer's site (carryover effects) (according to Regulation (EC) No. 1831/2003, article 3.2). In 2022, 19 of such national requests for substance-use permission - not authorised at a community level - were assessed by the Dutch NRL-FA. The requests concerned, among others, nutritional, sensory and zootechnical additives and coccidiostats. In some cases, additional information was requested by WFSR, which mostly concerned further details on feed processing.

For these 19 national requests, it was also evaluated by the Dutch NRL-FA whether they concerned GMOs or additives produced by GMOs. In three cases, it was concluded that the applications indeed concerned GMOs or additives produced by GMOs. In those cases, it was evaluated whether there were specific human and animal safety concerns and whether the applicant had submitted enough information to assess these aspects. In a limited number of applications, supplementary information was requested. In 2022, no application was rejected due to GMO safety aspects.

6.2.2 Other scientific and technical support

Assistance was given on request of the contact persons of the Ministry of Agriculture, Nature and Food Quality (LNV) and the Netherlands Food and Consumer Product Safety Authority (NVWA) as support of the Dutch delegation in the Standing Committee on Plants, Animals, Food and Feed of the European Commission (SCoPAFF). Advice was given on the status of certain products concerning the scope of Regulation (EC) No 1831/2003 and feed additives to be withdrawn from the market. Specific advice was given on the status of the mineral products chabasite, cristobalite and leonardite, on N-acetyl-D-glucosamine, and on calcium nitrate. More elaborate advice was given on applying seaweed (as a methane reducer) in dairy feed and on various aspects of (the use of) coccidiostats.

7 National Reference Laboratory Genetically Modified Organisms (GMOs)

Coordinator: Theo Prins

7.1 Activities within the EURL-NRL network

7.1.1 Participation in EURL-NRL workshops

In 2022, the Dutch NRL participated in the Steering Committee meetings of the European Network of GMO Laboratories (ENGL) in February (web meeting) and June (web meeting), the 42nd ENGL Steering Committee on 24 February and the 43rd ENGL Steering Committee on 9 June via Webex (presentation on the Work Group DNAex). At these meetings, the 18th NRL Workshop and the 33rd ENGL Plenary meetings were prepared (29 and 30 September, Ispra, IT). At the NRL meeting, a presentation was given on 'Detection strategies at WFSR'. A presentation on 'Screening and quantification' was given at the ENGL meeting.

42nd ENGL Steering Committee Meeting. SANTE presented the recent developments on New Genomic Techniques (NGT) and GM presence in feed/ food additives/enzymes. The presence of Genetically Modified Microorganisms (GMMs) in feed/food additives and enzymes has been discussed with Member States (MS) at the Standing Committee on Plants, Animals, Food and Feed (PAFF Committee) on 20 September 2021. The discussion is still ongoing because different MS have divergent views on addressing the problem. The current Working Groups gave an update on their work, and the ENGL Annual Meeting 2022/NRL training/NRL workshop and ENGL annual meeting were prepared. The Secretary shared a draft mandate for a new Working Group on the detection of GM animals. Regarding GM rice imports from China, SANTE informed that positive samples continue to be detected in products imported from China and that control activities are therefore continuing.

43rd ENGL Steering Committee Meeting. The recent policy action on New Genomic Techniques (NGTs) was presented in the update from SANTE. The developments related to the GM presence in food/feed additives/enzymes will most likely be discussed with the member states and possibly the ENGL members in September. The current Working Groups gave an update on their work. New activities entailed an update on the detection of NGTs and a Working Group on GM animals.

NRL meeting. The recent policy action on New Genomic Techniques (NGTs), Fact-finding studies, and GM presence in feed additives and food enzymes were updated by SANTE.

JRC representatives summarised the activities of the EURL GMFF in 2022 regarding the validation of new maize events MON95379 and DP15635 and soybean DBN9004. The JRC proposed to send the laboratory data of the ring trials using (e-signed) PDF files instead of shipping them on CDs. This approach is more convenient, less time-consuming and saves shipping costs.

The JRC provided a summary of the ENGL Working Group activities WG-Seq, WG-DNAex, WG-MPR2, WG-GMM and WG-NMT.

The result of PT GMFF-21/02 T2 (Maize T25 in maize flour) organised in 2021 was again discussed. There was an effect of the mastermix used in the ddPCR assays on T25 maize and its endogene *hmg* in the AOCS CRMs and seed powder. T25 copy numbers highly depend on the supermix with/without dUTP. T25 is underestimated in ddPCR with 2 of 3 Bio-Rad supermixes and in Qiagen cdPCR with one mastermix tested. The 'supermix for probes without dUTP' is without Bias.

The results on GMFF-21/02 T1 (soybean MON89788 in meat pate) were published (Broothaerts et al., 2023).

An update was given on the comparative testing activities in 2022 (see section 7.1.3). GMFF-22/01 included a blank food material containing no GM event (T1). The T2 sample consisted of rapeseed meal containing Ms8 and Rf3 oilseed rape events. The speaker reported that in agreement with SANTE, the value of 0.93 m/m should be rounded to 0.9%; therefore, based on the EURL GMFF results, the sample in the PT is compliant, and no labelling is required. The competent authority should provide guidance on whether or not to sum for the stack Ms8 x Rf3.

The EURL GMFF reported on the extension of their GMO database with the 'JRC GMO-Matrix'. Here, the success rate between a detection method and its target was given.

The Netherlands presented 'Detection strategies at WFSR'.

ENGL plenary meeting. The ENGL plenary meeting was held on 30 September and was attended by two representatives of the Dutch NRL. The meeting started with a presentation on the European Animal breeders' perspective on the potential use of new genomic techniques in Animal Breeding programs, followed by a presentation on the application of genome editing in animals. Belgium gave presentations on the metagenomic characterisation of GMMs and NGS strategies to detect a single-point variation in NGT plants. Germany presented its work on multiplex dPCR for the detection of GM soybean events, which also touched upon the calculation of the Probability of Detection (POD). Here, the minimal copy number for reliable quantification was 42. In an overview of CEN and ISO activities, a roadmap and strategy were presented for the revision of EN ISO 21669, EN ISO 21570, EN ISO 21571 and EN ISO 24276: ISO/TC 34 /SC 16. It was decided to gradually revise these ISO standards. This will be done in ISO/TC 34 /SC 16/WG 14 'Genetically engineered content detection and quantification'. The envisaged time path is now EN ISO 21669 (2023), EN ISO 21570 (2025), EN ISO 21571 (2026) and EN ISO 24276 (2027).

7.1.2 Participation in working groups

The Dutch NRL is chair of the Working Group (WG) DNA extraction. Several online meetings were organised. Updates were given to the EURL, and it is the intention to finalise the report of the WG DNAex in 2023.

The Dutch NRL participated in the WG Minimum Performance Requirements Part 2 (MPR2). In 2021 a final version of the "Definition of minimum performance requirements Part 2" was prepared and sent to the steering committee to make it an official ENGL publication. The final version still needs to be released.

The Dutch NRL participated in the WG Genetically Modified Microorganisms. A first draft of the report, also containing recommendations for detection strategies for GMMs and a description of available tools for detection, was finalised in 2021. The final version still needs to be released.

The Dutch NRL participates in the WG NGT. The main task of this WG is to review existing literature and recent experimental evidence on the detection of plant products obtained by targeted mutagenesis or cisgenesis and to verify if the conclusions of the 2019 ENGL report are confirmed. In 2022, a first draft was

7.1.3 Participation in proficiency and comparative tests

Two EURL proficiency tests were organised in 2022. In the first PT (GMFF-22/01), a Bakery mix containing GM Maize (T1) and a Rapeseed meal containing GM oilseed rape (T2) were used as samples (see also Table 7.1). In the second PT (GMFF-22/02), a Multigrain bread mix containing GM maize (T1) and a Soybean powder containing soybean event CV127 (T2) were used as samples. The Dutch NRL participated in this PT with good results (Table 7.1). For GMFF-22/02, there is currently a preliminary report only.

Table 7.1 *Overview of PT results GMO detection in 2022.*

Proficiency test	Analyte	Matrix	z-score	ζ score
GMFF-22/01	blank	T1: Bakery mix	n.a.	n.a.
GMFF-22/01	GM oilseed rape event Ms8 GM oilseed rape event Rf3	T2: Rapeseed Meal	-0.98	-1.47
			-1.38	-1.68
GMFF-22/02	GM maize event DAS59122	T1: Multigrain bread mix	-0.5	-0.6
GMFF-22/02	GM soybean event CV127	T2: Soybean powder	0.0	-0.1

n.a. = not applicable (No GM present).

7.2 Assistance to official laboratories

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

7.3 Scientific and technical support to the competent authority

WFSR participated in discussions with the Ministry of Agriculture, Nature and Food Quality (LNV), the Ministry of Infrastructure and Water Management (IenW), the Ministry of Health, Welfare and Sport (VWS), the Netherlands Food and Consumer Product Safety Authority (NVWA) and other parties on issues regarding the Dutch views on the potential criteria for the pre-notification of NGT-products. This is an ongoing discussion.

On a monthly basis, bilateral meetings are held with the NVWA, regarding GMO food and China rice. Also, 'Domain-meetings' organised by WFSR allow discussion with NVWA, the Ministry of LNV and the Ministry of VWS.

7.4 Contacts with other NRLs

Contact with other NRLs in the EU occurred during the Steering Committee meetings, the NRL meeting, and the ENGL Plenary Meeting. Since 2017 WFSR is also the NRL GM Food and Feed for Ireland. Ireland finances the NRL activities for 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.

WFSR and the Federal Office of Consumer Protection and Food Safety (BVL, Berlin, Germany) developed the GMO database EUginius. Partners of the consortium are the NRLs AGES (Austria), IHAR (Poland) and IZS (Italy).

7.5 References

Broothaerts, W., Hidalgo, R. B., Buttinger, G., Seghers, J., Maretti, M., Robouch, P., Corbisier, P. (2023). Proficiency of European GMO control laboratories to quantify MON89788 soybean in a meat pâté matrix. Food Control, 145, 6.

8 National Reference Laboratory Residues of 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 for all four EURL domains.

In the EURL-NRL network, one or more workshops 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 2022, two EURLs organised individual workshops while the other two collaboratively hosted a joint workshop.

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 setup and discuss the outcome of the annual EU proficiency tests, and to present the EURL program and activities for the following 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 three workshops, the items presented and discussed are briefly summarised below:

EURL workshop CF [DGI-Byen, Copenhagen, Denmark; 28-29 June 2022]
A representative from WFSR attended the workshop in person.

- Presentation of **EURL-proficiency test** (PT) of multi-residue analysis of pesticides (169 mandatory and 52 voluntary pesticides) in **barley**. 160 laboratories participated and submitted their results. In total, 19 residues were present, of which 12 incurred residues (resulting from field applications), concentrations varied from 0.023 to 4.40 mg/kg. Three of the pesticides present (fenpicoxamid, mefentrifluconazole, and benzovindiflupyr) were analysed by less than 50% of the laboratories. Quantification was satisfactory for more than 90% of the laboratories for 10 pesticides. For the others, this percentage ranged from 72-88%. The interlaboratory variability (RSD_R) varied from 17% to 27%, with an average of 22%. As known from earlier PTs, there was no apparent relationship between RSD_R and concentration.

  The EURL shared news from the Commission, including the updated Multi-Annual Control Programme (MACP) for 2022-2024. For cereals, this includes oats, barley, rye, brown rice, and wheat. Three new pesticides have been added to the mandatory scope: cyanthraniliprole, metaflumizone, and sulfoxaflor. The working document (SANCO/12745/2013, rev. 13(1) 22-23 Nov 2021) on recommendations regarding the inclusion of several pesticides in the national monitoring programmes was presented. Newly added pesticides are azadirachtin, clopyralid, metaldehyde, oxymatrine, phosphane (phosphine), and pyrethrins.
- Three **technical presentations** by the EURL and NRLs were given. The EURL presented (i) an automated method for cleanup (µSPE) of rapeseed extracts, (ii) results of a study on pesticide transfer from cereals to beer and beer by-products, and (iii) an exploration of the occurrence of residues of co-formulants in tomato and cereals. Seven NRLs (NL, CY, CZ, DE, LV, LU and AT) shared their analytical methods, practical experiences and monitoring programmes for cereals and/or feed analysis.

Combined workshop EURL FV & SRM [University Almeria, Spain; 13-14 October 2022, hybrid]
A representative from WFSR attended the workshop in person, while several others attended virtually.

- Presentation of **EURL-proficiency tests** (PTs). In 2022, the EURL-FV organised three PTs.
  One on **quantitative multi-residue analysis of 211 pesticides** (plus an additional 43 voluntary, based on SANCO/12745/2013) in **tomato**. The PT was open for NRLs and OLs; altogether, 174 laboratories participated. In total, 18 pesticides were present in the range of 0.012 to 0.61 mg/kg. The majority of the laboratories (80-97%) measured and reported most pesticides, but a few were only reported by 61%, 59%, or even as low as 38% of the laboratories. Quantification was satisfactory for more than 90% of the laboratories for the different individual pesticides. The interlaboratory variability (RSD_R) varied from 13% to 27%, averaging 18%. The RSD_R appears to be constant over the past five years, indicating that the measurement uncertainties are more or less constant. The second PT concerned a 'special commodity' (considered more difficult for analysis), in this case **dried beans**, to be analysed for **215 pesticides**. In total, 17 pesticides were present. Levels ranged from 0.027 to 0.081 mg/kg. A total of 60 laboratories participated. Also in this PT, not all laboratories covered all pesticides (e.g. aclonifen only 68% of the laboratories). Acceptable quantitative performance was obtained by 88%-96% of the laboratories. A high dispersion was observed for dichlorvos. The RSD_R varied from 12% to 43% (average 19.5%), reflecting the higher complexity of the matrix. The third PT was **rapid screening analysis** (qualitative, quantitative optionally) of pesticides in **tomato**. In this case, no target list was given; laboratories had to detect **'any' pesticide** and report within 72 hours. In total, 22 pesticides were present (all between 0.03-1.1 mg/kg, mostly below 0.1 mg/kg), including several long-banned and some very recently authorised pesticides. Only one laboratory detected all 22 pesticides. Fifteen laboratories out of 63 detected 21 pesticides, indicating that it is still challenging to find 'any' pesticide in a sample. 8-hydroxyquinoline was only detected by 11% of the laboratories.
- The **EURL-SRM** organised one PT and included **17 mandatory and 17 voluntary pesticides** that are troublesome in MRM methods, or can only be analysed using a dedicated SRM method. In total, 18 pesticides were present in the samples, including captan, THPI, dithiocarbamates (as CS_2), folpet, phthalimide, and maleic hydrazide. A total of 123 laboratories participated. For folpet/phthalimide, captan, ETU and methyldinocap, high variability of results was observed, which was attributed to their instability. The interlaboratory variability (RSD_R) varied from 17% to 44%, with an average of 28%, which is higher than for the multi-residue methods. This reflects the higher degree of difficulty.
- Nine **technical presentations** by the EURLs and several NRLs were given.
  EURL-FV conducted multiple presentations. One focused on the increase in throughput of LC-MS/MS analysis by dual-channel chromatography. Another discussed the use of ascorbic acid to stabilise certain pesticides in sample extracts. Attendees also learned about a comparison study for two cleanup approaches for multi-residue methods (dSPE vs µSPE) and an investigation on extraction issues with several dry matrices (dry pulses, soybeans, peanuts). Finally, there was a presentation on a comparison study on the use of supercritical chromatography coupled to tandem mass spectrometry (SFC-MS/MS) with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [also described in ABC 413 (2021) 5849-5857].

  EURL-SRM shared the experiences with routine control of herbs and spices. These are complicated matrices to analyse ('dirty' extracts, signal suppression, interferences) and frequently contain various pesticide residues.

  Three instrument suppliers proposed potential solutions to the helium shortage (gas often used in gas chromatography-based methods). Both gas-saving options and alternative carrier gases (especially hydrogen) were possible solutions.

  In the domain of single residue methods, ANSES (NRL France) shared the outcome of a French interlaboratory study of a method for the selective analysis of different classes of dithiocarbamates.

  The Dutch and North Irish NRL presented their experiences with columns and methods for the determination of highly polar and anionic pesticides (e.g. glyphosate and metabolites, perchlorate, ethephon etc.).

  NIBIO (Norway) shared their experiences with new generations of GC-MS/MS and LC-MS/MS equipment.

EURL workshop AO [CVUA Freiburg, Germany; 25-26 October 2022]
The workshop was attended in person by one representative from WFSR.

- Presentation of **EURL-proficiency test** (PT) of multi-residue analysis of pesticides (115 mandatory and 24 voluntary pesticides) in rape seed oil. 111 laboratories participated and submitted their results. In total, 22 residues were present, and concentrations varied from 0.032 to 0.64 mg/kg. Quantification was satisfactory for more than 90% of the laboratories for the 20 pesticides. For the others, this percentage was ranging from 75-89%. The interlaboratory variability (RSD_R) varied from 12% to 43% (pyridalyl), with an average of 20%. In 2022 also a dedicated PT was organised on lower levels of pesticides in baby food (infant formula, milk-based), 44 mandatory and 32 voluntary pesticides. 47 laboratories submitted results. In total, 15 pesticides were present in the range of 0.013 to 0.16 mg/kg. RSD_Rs varied from 10 to 24%, all below the harmonised 25% value. Quantitative performance was generally satisfactory, while higher percentages of questionable or unacceptable results were observed for benzalkonium chlorides (BACs) and chlorate.
- News from DG SANTE: the lack of analytical standards of pesticide metabolites included in the residue definition was commented on. If not commercially available, these standards should be supplied by the notifier of the pesticide. An update was given on the MACP and SANCO/12745/2013 (see also above) and on guidance documents dealing with processing factors (responsibility of the competent authority to decide if and which PF to use) [SANTE/2021/10704], on the evaluation of extraction efficiency [SANTE/2017/10632, rev.4], and on residue analytical methods for risk assessment and post-approval control and monitoring purposes [SANTE/2020/12830 rev. 1]. Furthermore, guidance values will be established for chlorate, BAC and DDAC in fish, as pesticide MRLs have not yet been set for fish. Several current challenges were mentioned, which included cumulative risk assessment (mixtures of pesticides), the review of MRLs for glyphosate (on hold until further clarity on its renewal) and the finding of matrine (besides being a pesticide used in, e.g. China, it also occurs as natural plant component).
- The EURL and NRLs delivered multiple technical presentations. The EURL presented on general challenges in products of animal origin, the long-term stability of pesticides in AO products (storage time, matrix, and pesticide-dependent), the footnotes for milk and egg (adjustment to percentage of fat), the implications of Regulation 2022/1644 for veterinary drugs that now also includes pesticides as illegal substances (this causes legislative conflicts), and residues of chlorate, BAC, DDAC and ethoxyquin found in fish. The Technical University of Denmark (DTU) revealed the issue of the multiple deltamethrin isomers (two trans isomers, and two cis isomers, which can all be separated). The applied product is one of the cis isomers. After application and during sample analysis, the second cis isomer can appear. Both should be summed for calculation and MRL compliance testing. PhD work on the automatisation of cleanup procedures and assessment of processing factors for meat products was presented. The NRLs of Cyprus, Croatia and Belgium shared their analytical methods, practical experiences and monitoring programmes of pesticides in products of animal origin.

In 2022, two co-workers from the pesticides teams of WFSR attended training courses organised by the EURLs. One was organised by the EURL-CF at DTU in Copenhagen (22-25 November 2022), focusing on automated cleanup (µSPE). The other was organised by the EURL-SRM at CVUA in Stuttgart (7-8 December 2022), addressing a variety of single residue methods.

8.1.2 Participation in working groups

WFSR is a member of the advisory group on proficiency tests organised by the EURLs and of the analytical quality control-working group for the bi-annual revision of the AQC document. In 2022, a meeting was held and attended on 21-22 June in Madrid, Spain. WFSR also participated in the CEN working group on standardising methods for pesticide residue analysis (CEN/TC 275/WG3 Pesticides).

8.1.3 Participation in proficiency and comparative tests

The NRL participated in 15 proficiency tests on pesticides in fruits/vegetables, cereals, products of animal origin, feed and other food matrices. This included PTs organised by the EURLs mentioned above and PTs from commercial suppliers. An overview is given in Table 8.1. A total of 170 z-scores (indicators for quantitative performance) were obtained. In general, the performance of the NRL was satisfactory (164 out

of 170), and the pesticides were correctly identified and quantified. A questionable result was obtained for four pesticide/matrix combinations (within the statistically expected range, but appropriate follow-up actions were taken). The result was unsatisfactory in two cases, triggering a root cause analysis. In one case, the deviation was due to erroneous reporting (reporting pesticides out of the method's scope). Where appropriate, corrective actions were taken.

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

PT Organiser			Performance [1]		
and PT code	**Matrix**	**Analytes[2]**	**S**	**Q**	**U**
EUPT AO17	rape seed oil	MRM pesticides	23	1	
EUPT CF16	Barley	MRM pesticides	11	1	
EUPT FV24	tomato	MRM pesticides	17		
EUPT SC05	dry beans	MRM pesticides	15		1
EUPT SRM17	tomato	SRM pesticides (bifenazate, chlorothalonil, dithiocarbamates (CS2) cyromazine, emamectine, maleic hydrazide, pymetrozine)	8		
Fapas 9143	wheat flour	SRM pesticides (glyphosate, chlormequat, mepiquat)	3		
Fapas 9146	Oats	SRM pesticides (glyphosate, AMPA, chlormequat, mepiquat)	4		
Fapas 19326	Honey	MRM pesticides	9		
Fapas 19329	pumpkin	MRM pesticides	26		
Fapas 19334	Lemon	MRM pesticides	14		
Fapas 19341	peas with pod	MRM pesticides	13	1	
Fapas 19347	Basil	MRM pesticides	11		1
TestQual 155	white cabbage	dithiocarbamates (CS2)		1	
TestQual 158	Wine	MRM + SRM pesticides (fosetyl, glyphosate, phosphonic acid)	9		
TestQual 171	Grape	dithiocarbamates (CS2)	1		

[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, three laboratories perform official analyses. This assignment is for a particular subdomain in all three cases: one for dairy products, already been acting for many years. The other two laboratories were assigned in 2021, specifically for organic products. For the dairy laboratory, in the frame of a quality control program, one sample of milk powder (a blind sample taken from a previous proficiency test) was sent for the determination of organochlorine pesticides. Results were reported to and evaluated by the NRL. Feedback from the NRL was provided to the OL. Quality control for the other two laboratories is done by reviewing their planned participation in proficiency tests and evaluating their performance and follow-up actions in case of deviating results. Both laboratories participate annually in a sufficient number of proficiency tests. In 2022, one laboratory was visited to discuss the follow-up and to share experiences of pesticide residue analyses in more general.

8.2.2 Advice

Other than in the quality control frame mentioned under 8.2.1, no specific advice was given.

8.3 Scientific and technical support to the competent authority

The Dutch NRL participates in regular (approx. every six weeks) meetings with the competent authority on official analysis. Additional meetings are held where needed. Specific topics addressed in 2022 were ethylene oxide, the increasing number and variety of import samples, the growing number of complex residue definitions (inclusion of metabolites), and the tendency to lower the MRL below the default 0.01 mg/kg for specific pesticides. At the competent authority's request, WFSR participated in the meeting of the Working Group for the Monitoring of Pesticide Residues on 17 October 2022.

8.4 Contacts with other NRLs

Through EURL workshops and CEN meetings on pesticides, the Dutch NRL connected with other NRLs. Moreover, the Dutch NRL attended the European Pesticide Residue Workshop (EPRW) in Bologna, Italy and Recent Advances in Food Analysis (RAFA) in Prague, Czech Republic, in September 2022. During both events, the Dutch NRL delivered an oral presentation and presented several posters.

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

Coordinator: Hanneke Brust

9.1 Activities within the EURL-NRL network

9.1.1 Participation in EURL-NRL workshops

The Technical University of Denmark (DTU) hosts the EURL for Metals and Nitrogenous Compounds. In 2022, the EURL organised the annual workshop and two trainings. A training was organised on determining inorganic arsenic in feed and food, and a second training was organised on nitrite and nitrate in feed and food.

The Dutch NRL did not attend the training on inorganic arsenic since it has extensive experience with the determination of inorganic arsenic, and the training was intended for NRLs with little or no experience. The training on the determination of nitrite and nitrate in feed and food was organised on the 4th and the 5th of October 2022 at the DTU and was attended by the Dutch NRL. The training focussed on the determination of nitrate and nitrite by ion chromatography with ultraviolet detection (IC-UV). The Dutch NRL is currently using a continuous flow analyser (CFA) for the determination of nitrate and nitrite in food. Both techniques are represented in standardised international methods (NEN-EN 12014-2:2018 and NEN-EN-ISO 12014-7:1998). Still, the HPLC/IC method is considered more sensitive and more commonly used due to the availability of the equipment in laboratories. The Dutch NRL does not have a method for the determination of nitrate and nitrite in feed but will be working on developing a (HPLC/IC-UV) method for feed in 2023.

The annual EURL workshop was held in Copenhagen on 16 and 17 November 2022. After two years of online or hybrid meetings, this was the first in-person workshop after COVID. There were thirty-six participants. Optionally, the workshop could also be attended virtually as "online observers". This was done by thirty-one participants. In total forty-five NRLs were represented at the workshop. The Dutch NRL attended the workshop in person.

Proficiency tests (PTs) organised by the EURL and issues associated with these PTs are usually extensively discussed during the workshop. In 2022, the EURL organised three different PTs:

- The first PT concerned the determination of total arsenic, cadmium, mercury, lead and inorganic arsenic in pet food (feed). The network's performance was very good for all parameters, although the number of laboratories (15) that submitted results for inorganic arsenic was relatively low compared to the other parameters (~35). The Dutch NRL submitted results for all five parameters. The inorganic arsenic content in the sample was below the limit of quantification (LOQ); hence no z-score could be calculated. Z-scores for the other four parameters were acceptable. The LOQ of the Dutch NRL for inorganic arsenic is relatively high compared to other laboratories. In 2023 the inorganic arsenic method will be optimised to obtain lower LOQs.
- The second PT covered the determination of total arsenic, cadmium, mercury, lead and inorganic arsenic in marine-based food (canned crab). The performance of the network was good for all parameters. Again no z-score for inorganic arsenic could be calculated for the Dutch NRL because the value was reported below the LOQ. The other z-scores were satisfactory.

- The third PT organised by the EURL concerned the determination of aluminium, total arsenic, cadmium, lead, and nitrate in vegetable-based food (spinach powder). The discussion focussed on the compliance assessment of dried foodstuffs with maximum limits on fresh products. The moisture content should be taken into account, and the concentration of the analytes should be calculated to fresh weight for compliance assessment. However, in practice, information on the moisture content of samples is not always available. The use of databases (such as the Dutch NEVO-online) was considered to be suitable in these cases. The determination of aluminium was discussed as well. During previous EURL workshops, it was noted that the measured aluminium content depends on the digestion temperature used. Lower aluminium recoveries were observed with digestion temperatures below 220 °C. If all laboratories use lower digestion temperatures, acceptable z-scores do not necessarily mean that labs perform well.

Other topics discussed at the EURL-NRL workshop included:

- *News from the Commission*. DG SANTE presented recent and future developments in the regulations for metals and nitrogenous compounds in feed and food. At the beginning of 2023, new maximum limits (MLs) for (inorganic) arsenic in food will be published. Currently, MLs for inorganic arsenic are only established for rice, but the regulation will be expanded with additional matrices such as babyfood, fruit juice, salt and additional rice products. The new MLs will be considerably lower than the current MLs, requiring lower LOQs. Therefore, the regulation on requirements for analytical methods for elements in food (EG 333/2007) has also been adapted. New MLs were also proposed for inorganic arsenic in fishery products, but because the drafted MLs are considered to be too low, the discussion on MLs for fishery products is still ongoing. The Dutch NRL's method for inorganic arsenic is currently not validated and accredited for the additional matrices and will therefore be adapted in the beginning of 2023. For mercury, MLs in fish were lowered in 2022, and a new ML was set in salt. In addition, a recommendation on monitoring total mercury and methylmercury in fish, crustaceans and molluscs was published in 2022. EFSA will perform a risk-benefit analysis for fishery products regarding the presence of contaminants versus health benefits. Therefore, the Dutch NRL and the competent authority have agreed to monitor methylmercury in fishery products from 2023 onwards. New MLs are under discussion for metals in seaweed, on which EFSA is currently drafting a report on exposure, and for nickel in food and feed. MLs for cadmium in food were lowered in 2022, but some new MLs (nuts, fungi) seem too low and may therefore rise in the coming year.
- *N-nitrosamines*. The scope of the EURL-NRL network has been expanded with nitrogenous compounds in 2018. Since then, the work on nitrogenous compounds focussed on nitrite and nitrate. However, the EURL is now requested by the European Commission to start working on N-nitrosamines as well. N-nitrosamines are potentially carcinogenic and can be formed by nitrite metabolites reacting with secondary amines. This is especially an issue in processed meat products, but N-nitrosamines can also be detected in other foodstuffs. EFSA is currently drafting an opinion on the risk assessment of N-nitrosamines in food. The EURL presented its plans regarding work on N-nitrosamines for the coming years, including method development by the EURL in 2023, the training of NRLs and the organisation of proficiency tests on N-nitrosamines from 2024 onwards. The Dutch NRL has worked on N-nitrosamines in the past, but most other NRLs have no experience in analysing N-nitrosamines.
- *Determination of lead in game meat.* The Austrian NRL presented their work on the determination of lead in game meat. Game is usually shot with lead (containing) bullets, which poses posing a challenge in accurately determining lead levels in game meat. Bullet fragments are spread through the meat and can be present in the wound to a depth of up to 15 cm. Although the tissue around the wound should be cut away, bullet fragments may still remain in the remaining tissue. Although game meat is homogenised in the laboratories before analysis, the presence of bullet fragments leads to very variable analytical results. In the most extreme example, replicate analyses of the same sample (a deer) were shown to result in values ranging from 0.23 to 175 mg/kg. There are no European maximum limits for lead in game meat, only for meat from pigs, bovine animals, sheep and poultry (0.1 mg/kg). Austria has an action level for game meat set at 0.25 mg/kg. However, due to the variable distribution of bullet fragments in the tissue, reporting accurate lead levels in game meat is often impossible. Many other NRLs, including the Dutch NRL recognised this issue. Several analytical strategies for game meat were discussed.

9.1.2 Participation in working groups

In 2022, there were no working groups on EURL-NRL issues related to metals or nitrogenous compounds in food and feed 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 (see 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 potassium in milk powder (FAPAS 18108) and nitrate in fish (FAPAS 15162). For potassium in milk powder, the obtained result fitted with the performance characteristics of the method. Hence, no further action was needed. For nitrate in fish (FAPAS 15162) an unsatisfactory z-score of +7.6 was obtained. The statistics performed by the PT organiser were however questionable. Besides nitrate, the PT was organised for nitrite as well. However, due to the large spread in results from the different participating laboratories, no assigned value and z-scores could be calculated. An assigned value and z-scores were calculated for nitrate, but a large spread in results from the different laboratories was also observed. Of the 15 participating labs, 12 labs were assigned a z-score, of which only 4 labs had a z-score between -2 and +2. Results from the different laboratories ranged from <LOD (LOD not specified) to 67 mg $NaNO_3$/kg; the assigned value was set at 12.9 mg/kg and WFSR had reported a value of 23.6 mg/kg. In the PT report, it was recognised by FAPAS that nitrate and nitrite in meat matrices more often yield variable results. Because of the poor statistical evaluation and the acceptable results in other PTs for nitrate, no further actions were taken.

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

PT	Analytes	Matrix	z-scores
Fapas 07428	Ni, As, Cd, Hg, Pb	Animal feed (plant origin)	Between 0.1 and 1.0
Fapas 07430	As, Cd, Hg, MeHg	Canned fish	Between -0.3 and 0.4
Fapas 07437	Cd, Pb	Honey	-0.3 and -0.4, resp.
Fapas 18108	Se, Na, Mg, P, K, Ca, Mn, Cu, Zn, Mo	Milk powder	Between -0.9 and 1.6; *2.1 for K*
Fapas 07440	Cu, As, Pb	Vegetable oil	Between -0.2 and 0.3
Fapas 07441	As, Cd, Hg, Pb	Purée of root vegetables	Between 0.0 and 0.5
Fapas 07446	As, Cd, Hg, Pb	Offal (liver)	Between -0.2 and 0.2
EURL-MN PT-2022-03	Al, As, Cd, Pb, nitrate (NO_3)	Vegetable-based food (spinach)	Between -0.68 and 0.86
EURL-MN PT-2020-01	As, Cd, Hg, Pb	Pet food (feed)	Between -0.34 and 0.75
EURL-MN PT-2020-02	As, Cd, Hg, Pb	Marine-based food	Between -0.33 and 0.39
Fapas 15159	Nitrate (as $NaNO_3$) and nitrite (as $NaNO_2$)	Milk powder	1.5 and 1.3, resp.
Fapas 15160	Nitrate (as $NaNO_3$) and nitrite (as $NaNO_2$)	Meat	0.1 and 1.7, resp.
Fapas 15161	Nitrate (NO_3)	Cabbage puree	-0.1
Fapas 15162	Nitrate (as $NaNO_3$)	Fish	*7.6*
Fapas 30121	Melamine and cyanuric acid	Infant formula	-0.3 and 0.1, resp.

9.2 Assistance to official laboratories

9.2.1 Quality control

Analyses for the competent authority for milk and milk products are carried out by an OL. The Dutch NRL investigated the quality of the OL assigned by the competent authority for the analyses of metals and minerals. This investigation included a test round with samples with a known metal content, CRM materials

or spiked samples sent to the OL and routine samples from the OL that the Dutch NRL reanalysed. The results of the analyses by the OL were discussed with the competent authority for milk and milk products and reported.

Additionally, two OLs were appointed in 2021 to analyse metals in biological products. The quality of both OLs was investigated by evaluating the PT results of both labs.

9.2.2 Advice

Advising the OL for milk and milk products is on an ad-hoc basis and depends on the outcome of the comparison test organised two times a year by the NRL. In 2021 no specific advice was given based on the outcome of the comparison test. On request, one of the OLs for biological products was advised on interpretation of the legislation concerning maximum limits of processed foods. In addition, this OL was visited by the Dutch NRL on March 31, together with the NRL for pesticides. The evaluation of the PT results, analytical methods and method validation were discussed, and information from the EURL-NRL network was shared with the OL. Specifically, advice was given to the OL on performing validation studies on their methods for the analysis of metals. A visit to the other OL for biological products is planned for the beginning of 2023.

9.3 Scientific and technical support to the competent authority

In 2022, there were several contact moments between WFSR and the competent authority. Specifically, the NRL provided information on the possibilities of arsenic and mercury speciation to the competent authority.

9.4 Contacts with other NRLs

During the EURL workshop, relationships with other NRLs were maintained. Information was exchanged on analytical methods and experiences with other NRLs. In addition, there was contact with other NRLs through a CEN meeting (online) of the working group on elements and their chemical species.

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

Coordinator: Marta Sopel

10.1 Activities within the EURL-NRL network

10.1.1 Participation in EURL-NRL workshops

On 4 and 5 October 2022, a fifth Workshop of the European Union Reference Laboratory (EURL) for mycotoxins & plant toxins in food and feed was organised by WFSR in the Netherlands. It was attended in person by the representatives of the Commission, the Netherlands Food and Consumer Product Safety Authority (NVWA), WFSR scientists of the EURL, representatives of NRLs and one guest from Brazil; two Member States were present online.

The workshop agenda comprised an update of relevant legislation and CEN activities, results from proficiency tests (PTs) organised by the EURL, BfR and DG TAXUD, discussions on upcoming legislation on sampling and analysis of mycotoxins and plant toxins and on method development. A summary of the topics presented and discussed during the Workshop is given below:

European Commission update on legislation and emerging issues, based on the presentation by Frans Verstraete from Directorate-General for Health & Food Safety.

- *Mycotoxins in food*. Maximum levels (ML) for ergot alkaloids as in Commission Regulation (EU) 2021/1399 are applicable since 1 January 2022. Maximum levels of ochratoxin A in certain foodstuffs were amended by Commission Regulation (EU) 2022/1370. MLs in several matrices are under discussion: for deoxynivalenol (DON), new matrices added and MLs lowered, modified forms are considered; for T-2 and HT-2 toxins, new matrices added and MLs lowered to below indicative levels; indicative levels for *Alternaria* toxins are discussed. MLs for aflatoxins are to be evaluated for herbs, spices, cocoa, almonds, ice/desserts and gluten.
- *Mycotoxins in feed*. Commission Regulation (EU) 2022/1104 of 1 July 2022 amended Regulation (EU) No 68/2013 on the Catalogue of feed materials.
- *Plant toxins in food*. Recent amendments on plant toxins cover MLs for pyrrolizidine alkaloids (Commission Regulation (EU) 2020/2040 of 11 December 2020), MLs of tropane alkaloids (Commission Regulation (EU) 2021/1408 of 27 August 2021), MLs of opium alkaloids (Commission Regulation (EU) 2021/2142 of 3 December 2021), MLs of hydrocyanic acid (Commission Regulation (EU) 2022/1364 of 4 August 2022), MLs of delta-9-tetrahydrocannabinol (Δ9-THC) in hemp seeds and products derived therefrom (Commission Regulation (EU) 2022/1393 of 11 August 2022). Recommendation on monitoring the presence of glycoalkaloids in potatoes and potato-derived products was given – (Commission Recommendation (EU) 2022/561 of 6 April 2022). Quinolizidine alkaloids are under discussion.
- *Plant toxins in feed*. Indicative MLs are under discussion for maximum levels for Δ9-THC in feed, tropane alkaloids, pyrrolizidine alkaloids, quinolizidine alkaloids and hydrocyanic acid in linseed for birds.
- *Sampling and analysis*. EURL contaminants network is preparing a guidance document for analytical quality control (AQC) aspects of the legislation on sampling and analysis for plant toxins and performance criteria confirmatory methods.

- *Control plan contaminants food*. Regulation (EU) 2017/625 (as of 14 December 2022) repeals the Council Directive 96/23/EC, which covers a control plan to monitor residues of pharmacologically active substances, pesticides and contaminants in animals and food of animal origin. Commission Delegated Regulation (EU) 2022/931 of 23 March 2022 supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council by laying down rules for the performance of official controls on contaminants in food. Commission Implementing Regulation (EU) 2022/932 of 9 June 2022 on uniform practical arrangements for the performance of official controls of contaminants in food, on specific additional content of multi-annual national control plans and specific additional arrangements for their preparation shall apply from 1 January 2023.
- *Review of Regulation (EC) 152/2009*. A procedure for macroscopic analysis, (possible) reference to EN standards and analytical performance criteria for feed are under discussion.

CEN activities on mycotoxins in feed and glucosinolates (GSL), based on the presentations from NVWA and WFSR, CEN.

- *CEN activities food*. Within the technical working group TC275 on food, WG5 on biotoxins, current work in Mandate 520 focuses on method development: Multimethod for the determination of aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone by LC-MS/MS (EN 17641 Foodstuffs – published). Possible topics for new Standardization requests are: atropine and scopolamine – herbal infusions, millet, sorghum, maise, buckwheat (Commission Regulation (EU) 2016/239 and Commission Regulation (EU) 2021/1408), hydrocyanic acid – extension with linseed, almond, cassava (Commission Regulation (EU) 2017/1237 – (EU) 2022/1364), erucic acid (Commission Regulation (EU) No 696/2014 – (EU) 2019/1870), pyrrolizidine alkaloids (Commission Regulation (EU) 2020/2040), ochratoxin A – extension of matrices ((EU) 2022/1364), THC in food (Recommendation (EU) 2016/2115 – (EU) 2022/1393), opium alkaloids (Recommendation 2014/662/EU – (EU) 2021/2142, quinolizidine-alkaloids and glycoalkaloids (EFSA calls for data)).
- *CEN activities for feed*. Current work in Mandate 520 focuses on projects for T2/HT2, DON, ZEN by LC-MS/MS in cereals and complete feed (EN16877), ergot alkaloids and tropane alkaloids by LC-MS/MS in cereals and complete feed (EN17256), multi-mycotoxin method by LC-MS/MS (DON, AB1, FB1 and FB2, T2/HT2, ZEN, OTA) in cereal-based feed (EN17194), free gossypol by LC-MS in cotton/-based materials (EN17504), intact glucosinolates in feed ingredients/complete feed by LC-MS (FprEN 17853) and pyrrolizidine alkaloids in feed by LC-MS (prEN17683). Possible topics for a new mandate are: a method to assess the binding capacity of registered binders for aflatoxins, THC in hemp-derived feed materials (technique to be chosen), extension of scope EN17194 (conjugates and emerging mycotoxins), assessment RSD_R's of existing CEN methods (consequence of feed/myco criterion document), cyanogenic glucosides (HCN through determination as intact CNGs by LC-MS), quinolizidine alkaloids (lupin seeds) and *Alternaria* toxins in feedstuffs.

Proficiency tests, based on the presentations given by EURL, BfR Germany and DG TAXUD.

- *CLEN PT on cannabinoids*. In 2022, a ring test on cannabinoids was organised among the Customs Laboratory European Network (CLEN) and interested laboratories. Participants were provided with 6 samples (3 matrices, with low and high contents in cannabinoids): 2 dried powdered flowers, 2 CBD in MCT oils (spiked) and 2 cookies in powder. The participants were asked to quantify: THC (Δ9-THC), THCA, total THC, CBD, CBDA and total CBD, cannabinol, cannabigerol and cannabigerolic acid. Forty-nine out of fifty laboratories registered for the PT reported results. For the sum of THC and THCA in the samples with high content, z-scores were satisfactory for 91%, 92% and 96% of the participants for dried flower, CBD oil and cookies, respectively. For total CBD, z-scores were satisfactory for 91%, 79% and 97% of the participants for dried flower, CBD oil and cookies, respectively.
- *PT on Alternaria toxins*. The PT on *Alternaria* toxins was organised in September 2021. Each participant received one sample of sunflower seeds and one mixed control standard (thin film). The participants were asked to quantify: alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and additionally tentoxin (TEN) and altenuene (ALT). A total of 13 laboratories participated, and the results were presented at the meeting. Twelve participants reported results for AOH, AME, TeA and TEN in the sunflower seeds sample and only 2 for ALT. Four participants reported results with deviating z-scores. Among these participants, three of them used an in-house developed analytical method, and one used the CEN method. In the case of deviating z-scores, the reason mentioned was problems with the analytical standard.

- *PT on multi mycotoxins*. The EURL mycotoxins & plant toxins organised a PT on multi-mycotoxins in cereal-based food and feed in 2021. The results of this PT were processed and reported in 2022. A total of 45 laboratories participated. Participants received one sample of oats and one sample of maise. They were asked to quantify 6 mycotoxins with legal limits in food: deoxynivalenol (DON), fumonisin B1 (FB1), fumonisin B2 (FB2), T-2 toxin, HT-2 toxin and zearalenon (ZEN). Quantification of 10 non-regulated mycotoxins was voluntary: 3-acetyl-deoxynivalenol (3-Ac-DON); 15-acetyl-deoxynivalenol (15-Ac-DON); deoxynivalenol-3-glucoside (DON-3-G); nivalenol (NIV); alternariol (AOH); alternariol monomethyl ether (AME); enniatin A (Enn-A); enniatin A1 (Enn-A1); enniatin B (Enn-B) and enniatin B1 (Enn-B1). Forty participants submitted results for all 6 mandatory mycotoxins, four participants submitted results for all of the 10 voluntary mycotoxins, and twenty-four participants submitted results for 1 to 9 of the voluntary mycotoxins. In general, the performance for the mandatory mycotoxins was satisfactory, with a point of attention for false negative results (5x) and false positive results (14x fumonisins). For several voluntary mycotoxins, the performance was satisfactory; for others, the results varied largely, with apparent outliers; a low number of results complicated the evaluation. Further improvement of the method performance amongst NRLs (EURL) was advised.
- *PT on pyrrolizidine alkaloids*. As a follow-up to the Research Study organised in 2019 (EURL PT-02), a PT on pyrrolizidine alkaloids (PAs) was organised in 2022. The participants were asked to analyse two samples: dry black tea and marjoram, each for 35 PA (21 PAs and 14 PA isomers). Only 24 participants subscribed, even though the new legislation came into force on 1 July 2022. The z-scores for the individual PAs (total score of 27) and the sum of the PAs (total score of 2) were evaluated. Most participants had all 35 PAs included in the method at the required LOQ, but the $RSD_{R's}$ were relatively high, and for black tea, all were above the 25% target.
- *Revision of Regulation (EC) No 401/2006: performance criteria mycotoxins and plant toxins in food & feed, based on the presentations given by WFSR, EURL.* The most relevant modifications in the 401/2006 were emphasised, and some parts of the text need rephrasing. The same sampling strategy and method performance criteria will be included in implementing regulations for plant toxins. In breakout groups, the practices used by various laboratories regarding correction for recovery were discussed. There is no single fixed procedure: the correction is done using the average of the initial validation, the average of the ongoing validation, and/or the recovery obtained from the current analysis of samples. These multiple options may be included in the EURL guidance document for information purposes.
- *Glycoalkaloids in boiled potato, given by WFSR.* Glycoalkaloids (GAs) are inherent plant toxins of *Solanaceae* plants such as potato, aubergine and tomato. Acute toxic effects may occur following ingestion of total potato GAs of 1 mg/kg body weight (b.w.) or more, and doses in the range of 3–6 mg GAs/kg bw are considered potentially lethal for humans. A level below 200 mg/kg in whole raw potato is regarded as safe but may still lead to toxic effects. Thus EFSA advises an indicative maximum level of 100 mg/kg as the sum of α-solanine and α-chaconine in potatoes and processed potato products. A method for GAs in boiled potato was developed and validated to conduct the survey and estimate the reduction of GAs by peeling and boiling. The preliminary results showed that, on average, GAs were reduced by 77% by peeling the potato, and subsequent boiling reduced the GA concentrations by a further 6% compared to the raw, whole potato.
- *Method development quinolizidine alkaloids by WFSR, EURL.* Quinolizidine alkaloids (QAs) occur in plant species of the Fabaceae family, such as white, blue, yellow and Andean lupin. QAs like sparteine, angustifoline and multiflorine have teratogenic properties; however, the data on their toxicity, transfer, and occurrence are limited. A method for 13 QAs in lupin seeds, lupin-based meat imitates, including milk imitates, and bovine milk using LC-MS/MS, was successfully developed and validated.
- *Ochratoxin A in cured meat and cheese by WFSR, EURL.* More information is needed on the exposure to OTA via cured meat and cheese. Thus a method for the analysis of OTA in these matrices at an LOQ of 0.2 µg/kg was developed and validated in 2022. The sampling procedure included sub-sampling inside versus outside of the products, as OTA produced on the outside of the product migrates towards the inner side of the product. The method will be published on the EURL MP website and will be used for a survey in cured meats in 2023.
- *Detection of mycotoxins in beer with multiplex microsphere immunoassays by WFSR.* WFSR presented the method developed to measure mycotoxins in beer using sensors.

10.1.2 Participation in working groups

As a result of discussions within the working group on analytical quality control (AQC), initiated by WFSR as part of the EURL task, a "Guidance document on performance criteria" drafted by the EURL has been published and is available on the EURL-MP website.

10.1.3 Participation in proficiency and comparative tests

In 2022, the Dutch NRL participated in 19 proficiency tests (PTs): 16 PTs on mycotoxins, 1 PT on pyrrolizidine alkaloids and tropane alkaloids, 1 PT on tropane alkaloids and 1 PT on cannabinoids, of which the overview is presented in Table 10.1. For the 142 toxin/matrix combinations, 124 z-scores were obtained, of which 111 were satisfactory. Five questionable results were obtained in four PTs: aflatoxins B1, G1 and the sum of aflatoxins in peanuts; aflatoxin G1 in rice and heliosupine N-oxide in dried nettle. Three unsatisfactory results were obtained for the sum of α+β ergocryptine in rye flour and THCA in dried powdered flowers, and CBD in MCT oil. All deviations were analysed using a root cause analysis. For FAPAS 04436, a study with CRM-certified Mycotoxins standard solutions has been carried out. A number of Mycotoxins have a small deviation in the standard solutions used, up to 10%. The concentrations of Aflatoxins B1 and G1 have been adjusted downwards, and the concentrations of Aflatoxins B2 and G2 upwards. For Aflatoxin G1 in rice, the reanalysis of the sample gave a good z-score. The deviation could possibly be caused by the applied recovery correction based on historical data and not on standard addition. Currently, the standard addition method is applied to samples in which more than 1.5xML to 2.5xML is found at the first analysis. For FAPAS 22190, the deviation concerns the sum of two ergot alkaloids (EAs), which are measured separately but could only be reported as a sum. There appeared to be a fairly large duplicate difference in the calculated concentrations. The reported sum of EAs has a z-score of 0.44 and therefore meets the requirements. For heliosupine N-oxide, the ring test sample was re-analysed with satisfactory results. For the ring test on cannabinoids, the reanalysis showed that the contribution of the interference decreases at 270 nm.

Table 10.1 *Overview of proficiency tests the Dutch NRL Mycotoxins and plant toxins participated in.*

PT Organiser and PT code	Matrix	Analytes[2)]	Performance [1)]		
			S	Q	U
FAPAS 04412	Baby food	AFLA B1, B2 AFLA B1+B2, DON, OTA	1	0	0
FAPAS 04420	Sesame paste	AFLA B1, B2, G1, G2, AFLA sum, OTA	5	0	0
FAPAS 04432	Pistachio	AFLA B1, B2, G1, G2, AFLA sum	5	0	0
FAPAS 04433	Maize	AFLA B1, AFLA sum, DON, OTA, ZEN	4	0	0
FAPAS 04436	Peanut	AFLA B1, B2, G1, G2, sum	2	3	0
FAPAS 04439	Maise	AFLA B1, B2, G1, G2, AFLA sum, OTA, DON, FB1, FB2, FB1+FB2, ZEN, T-2, HT-2, T-2+HT-2	10	0	0
FAPAS 04441	Almond	AFLA B1, B2, G1, G2, AFLA sum, OTA	6	0	0
FAPAS 04447	Pet Dog Food	AFLA B1, FB1, FB2, FB1+FB2, T-2, HT-2, T-2+HT-2, DON, ZEN, OTA	10	0	0
FAPAS 04449	Dried figs	AFLA B1, B2, G1, G2, AFLA sum, OTA	6	0	0
FAPAS 04452	Chilli powder	AFLA B1, B2, G1, G2, AFLA sum, OTA	2	0	0
FAPAS 04459	Rice	AFLA B1, B2, G1, G2, AFLA sum	4	1	0
FAPAS 17219	Coffee	OTA	1	0	0
FAPAS 17226	Coffee, green	OTA	1	0	0
FAPAS 22190	Rye flour	EA, total	12	0	1
FAPAS 22198	Baby food	TA	2	0	0
PROOF-ACS P2203-RT	Dried nettle	PA, TA	11	1	0
BfR	Cheese	OTA	2	-	-
Sciensano Multi-mycotoxin 2022	Maise flour	AFLA B1, B2, G1, G2, AFLA sum, FB1, FB2, FB3, FB sum, DON, OTA, ZEN, T-2, HT-2, T-2+HT-2, ENN A, A1, B, B1, ENN sum, BEA	11	0	0
BIPEA Ring test on cannabinoids - 2022	Dried powdered flowers CBD in MCT oil Cookies in powder	Δ9-THC, THCA, Total THC, CBD, CBDA, Total CBD, CBN, CBG, CBGA, Total CBG	17	0	2

[1)] number of toxins in the PT: S= satisfactory (z< |2|), Q = questionable (|2| <z< |3|, U = unsatisfactory (z> |3|).

[2)] AFLA= aflatoxin, OTA = ochratoxin A, DON = deoxynivalenol, FB = fumonisins, ZEN = zearalenone, ENN= enniatins, BEA= beauvericin, PA= pyrrolizidine alkaloids, TA= tropane alkaloids, EA= ergot alkaloids, Δ9-THC=Δ-9-tetrahydrocannabinol, THCA= tetrahydrocannabinolic acid, CBD= cannabidiol, CBDA= cannabidiolic acid, CBN= cannabinol, CBG= cannabigerol, CBGA= cannabigerolic acid.

10.2 Assistance to official laboratories

10.2.1 Quality control

Besides the NRL, which is also an Official Laboratory (OL), one other OL in The Netherlands conducts the analysis of milk and dairy products for the presence of aflatoxin M1. The quality control of this OL was done by sending standard solutions, quality control material, blank milk powder and milk samples containing aflatoxin M1 in two rounds.

10.2.2 Advise

The Dutch NRL provided feedback to the OL based on the reported results. The results were satisfactory, and OL's quality control should continue in its current form.

10.3 Scientific and technical support to the competent authority

Contacts with the competent authority are kept regularly through bilateral and national meetings. The expert working group meetings on agricultural contaminants were attended by the Dutch NRL, the Ministry of Health, Welfare and Sport (VWS), the Ministry of Agriculture, Nature and Food Quality (LNV) and the Netherlands Food and Consumer Product Safety Authority (NVWA). The technical aspects of detecting emerging contaminants were discussed.

10.4 Contacts with other NRLs

Contacts with other NRLs were maintained through the EURL-NRL workshop, CEN meetings on mycotoxins and plant toxins in food and feed, discussions of working groups on analytical quality control and the LNR TOX communication group. Additionally, the following symposia and conferences on mycotoxins and plant toxins were attended by the Dutch NRL: the 43rd Mycotoxin Workshop in Toulouse, France; the 13th conference of the World Mycotoxin Forum in Parma, Italy; the 10th International Symposium on Recent Advances in Food Analysis in Prague, Czech Republic and the 4th International Congress on food safety and quality in Dubrovnik, Croatia.

11 National Reference Laboratory Processing contaminants

Coordinator: Liz Leenders

11.1 Activities within the EURL-NRL network

11.1.1 Participation in EURL-NRL workshops

The Technical University of Denmark (DTU) hosts the European Union Reference Laboratory Processing Contaminants (EURL-PC). In 2022, the Dutch NRL participated in the annual workshop of the EURL hosted in two sessions on 27 and 29 September in Copenhagen, Denmark. Over 30 participants attended the event, representing the NRLs, the Directorate General Health and Food Safety (DG SANTE) and staff from the EURL-PC. The meeting started with an update from the Commission, followed by presentations from representatives of the EURL-PC and NRLs regarding their recent method development and discussions about the results of the PTs organised in 2022. The first session ended with a discussion concerning the implementation of the LOQ estimation guidance document and an update regarding the document for measurement uncertainty. On the second day, the first part of the scientific programme focused on a new scientific task within the processing contaminants domain starting from 2023: the mineral oil hydrocarbons (MOH). The session continued with a session in which the future needs for NRLs were discussed, and representatives from EURL-PC gave some presentations on their recent work on method development; the proposed PTs for 2023 were also presented. The workshop finished with a method validation training.

- *Update from the Commission*. DG SANTE presented recent and future developments of the EU policy and regulations for processing contaminants, which were quite similar to the developments in 2021. Discussions on the setting of maximum levels (MLs) for 3-monochloroprane-1,2-diol (MCPD) esters and glycidyl esters (GE) are still ongoing. Possible MLs complementary to the MLs established by the Commission Regulation (EU) 2022/1322 are considered and are based on the available data in the EFSA database and other factors, such as the specific vegetable oil content. In certain cases, significant divergences are observed between levels found in food and expected levels, taking the fat content into account. MLs are considered for a wide range of foods, such as margarines, instant noodles, cereal-based foods for infants and young children, baby food and potato crisps. The specific food categories have yet to be decided. After that, the MLs for these food categories will be discussed, and the approach for other foods will be enforced. Next, they presented the ongoing discussions on the review of existing benchmark levels, the establishment of new benchmark levels and the establishment of MLs for acrylamide. The existing benchmark levels for several food products are considered to be lowered, and a list of suggested MLs for these food products is shared. Suggested benchmarks for new food products, such as vegetable fries and cocoa powder, are also presented. Furthermore, the recommendation for analysis of furan and alkylated furans was presented (Commission Regulation (EU) 2022/495). Member States and food business operators should use this recommendation for the analysis of furans and alkylfurans. EFSA has identified a health concern related to furans and methylfurans (2-methylfuran, 3-methylfuran and 2,5-dimethylfuran) in food. Still, it is acknowledged that there are no sufficient occurrence data on alkylfurans (methylfurans, 2-ethylfuran and 2-pentylfuran) in certain foods to perform a reliable exposure assessment. It is vital that more data are collected from the NRLs; the recommendation should help the NRLs to set up a reliable method. Lastly, the Commission presented a new compound class to be included in the EURL-PC scope in 2023: mineral oil hydrocarbons (MOH). In 2023, EFSA will publish an updated risk assessment regarding MOH, after which discussions of a possible regulatory follow-up can be started (MLs for MOSH/MOAH in food). Meanwhile, under Article 14 of the General Food Law (Regulation (EC) No 178/2002), competent authorities in member states can take enforcement action against MOHs in food if a food is deemed unsafe. To ensure a uniform enforcement approach throughout the EU, the Member States agreed to withdraw and, if necessary, to recall products from the market when the sum of the concentrations of MOAH in food is at or above the following maximum LOQs: 0.5 mg/kg for dry foods with a low fat/oil content

(≤4% fat/oil); 1 mg/g for foods with a higher fat/oil content (>4% fat/oil); 2 mg/kg for fats/oils. Because of the importance of reliable analysis of MOH, particularly MOAH, the Commission has asked the EURL-PC to start working on the analysis of MOH in food.

- *Presentations on method development EURL-PC and NRLs*. A representative from a German NRL gave a presentation regarding their recent work concerning the status of method standardisation for MCPD and GE determination in compound foods. This presentation was followed by a series of presentations by the EURL-PC concerning the effects of manufacturing conditions on hydroxymethylfurfural (HMF), 4-methylimidazole (4-MI) and 2-acetyl-4-tetrahydroxybutylimidazole (THI) contents in caramel colours and the validation of the analysis method for these compounds, and a presentation about MCPDs and GE in plant-based and meat alternative products in which it was shown that free and bound MCPD and GE are found in plant-based and meat alternative products, but in rather low levels. Free MCPDs were found in seaweed samples in amounts that could be a significant source of exposure to free 3-MCPD if seaweed consumption becomes more mainstream. Furthermore, a presentation regarding the finding of PAH derivatives in smoked fish, the comparison of analytical methods for the determination of acrylamide and finally, the method development for the analysis of ethyl carbamate using arrow-SPME-GC-MS/MS.
- *Discussion of the EURL PTs performed in 2022*. The workshop continued with a discussion on the three PTs that the EURL organised in 2022. The first PT concerned the determination of furan and alkylated furans in cereal products. Since the analysis of furans is a relatively new subject, not all NRLs participated in this PT. Overall, the analysis of furans in cereal products showed good results for most laboratories. For furan and 2-methylfuran, most labs achieved good z-scores. For 3-methylfuran there was a wide range of reported values resulting in a very high uncertainty of the assigned value, although outlying results were compensated for. For 2-ethylfuran and 2,5-dimethylfuran only seven respectively four laboratories reported results, leading to only indicative z-scores for 2-ethylfuran and no z-scores for 2,5-dimethylfuran. The second PT concerned the determination of acrylamide in cereal-based infant food. Overall, the analysis of acrylamide in cereal-based infant food showed excellent results. Out of the 36 labs that participated in this PT, 32 were able to meet the LOQ criterion of ≤20 µg/kg set by the Commission Regulation (EU) 2017/2158. Lastly, the EURL-PC PTs for free and bound 2- and 3-MCPD and GE in powdered infant formula and baby biscuits were discussed. The results of this PT showed that there are still issues with the analysis of the compounds for some labs, both for bound and free forms of MCPDs, but there seem to be more issues with free forms of MCPDs (more questionable z-scores). However, this was the first time the EURL-PC organised a PT in which free MCPDs needed to be analysed, so future PTs need to confirm this conclusion.
- *Implementation of the guidance documents for LOQ estimation and measurement uncertainty*. At the end of the first day of the workshop, the guidance document for the estimation of the LOQ was presented and discussed. This document was written by the joint EURL for contaminants, consisting of the EURL-PC, the EURL for Persistent Organic Pollutants (POPs), the EURL for Metals and Nitrogenous compounds (MN) and the EURL for Mycotoxins and Plant toxins (MP). The work started in 2019 and has already been discussed in the EURL-PC workshops in 2020 and 2021. The document indicates a general approach to estimating the LOQ: the LOQ is the lowest validated level of an analyte, for which it has been demonstrated that the respective criteria for identification, precision and trueness are met. Any obvious hurdles for implementation, implementation in future method validations and ongoing validations, and any need for adjustments of old methods were discussed. Lastly, an update was given regarding the document on measurement uncertainty. In this document, it is stated that within the scope of EURLs for contaminants (including compliance testing) the measurement uncertainty associated with the analytical results reported in the analysis report, will not take into account the uncertainty from external sampling but only the uncertainty of the work performed by the lab itself.
- *New scientific task for NRLs: MOH*. Mineral oil hydrocarbons (MOH) are complex mixtures of hydrocarbons, derived from crude oil or produced from coal, natural gas or biomass through Fisher-Tropsch synthesis. MOH can be divided into two main types: mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). In 2019, EFSA published a rapid risk assessment regarding the possible risk to public health due to the contamination of infant formula and follow-on formula by MOAH. In 2023 an updated risk assessment will be published, most likely to be followed by MLs. In 2023, MOH will be added to the scope of the EURL-PC, and a MOH core working group/task force will be launched. The EURL-PC will provide training for the analysis of MOH in autumn 2023.

- *EURL-PC PTs in 2023 and NRLs' future needs*. A discussion session was held on the future needs of NRLs. The outcome was a wish from the NRLs regarding defining matrix food pools to be used when validating analytical methods, including examples of blank food samples. There was a general wish from the NRLs to the EURL-PC to offer hands-on or alternative training. Videos provided by the EURL-PC for online training during the Covid pandemic were recognised as helpful by many NRLs, although hands-on training is still preferable. Proposed PTs for 2023 were presented and are expected to include (1) 3-MCPD, 3-MCPD-esters and GE in a relevant matrix, e.g. baby foods, (2) determination of the content of acrylamide in cacao powder, since Benchmark Levels for acrylamide are suggested, and (3) determination of furan and alkylfurans in a sample of breakfast cereals, biscuits or crispbread, as suggested in the EU Recommendation. Finally, on MOH, a PT in a standard solution or a relatively easy matrix, e.g. rice, was proposed. Training to determine processing contaminants (for instance free 2- and 3-MCPD) will be offered to the NRLs in 2023.
- *Training Method validation*. To produce reliable results, laboratories must validate a method prior to use to prove that requirements have been met. The EURL-PC gave an overview of different parameters to validate, such as repeatability, trueness and LOD/LOQ. The performance criteria for selected contaminants in foodstuffs related to Commission Regulation 333/2007 were discussed, and the training ended with an exercise to calculate all parameters.

11.1.2 Participation in working groups

In 2022, there were no working groups on EURL-NRL issues related to processing contaminants in food to participate in. The EURL is discussing setting up such focussed working groups in future.

11.1.3 Participation in proficiency and comparative tests

The scope of the EURL and NRL in 2022 included PAHs, 2- and 3-MCPD esters and GE, acrylamide and (alkylated) furans. The Dutch NRL participated in PTs for all these processing contaminants. In 2022, the NRL participated in three PTs organised by the EURL; one of them included two different matrices to be analysed (EURL-PC 2022-11 and EURL-PC 2022-12). 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 for NRL Processing contaminants.*

PT	Analytes	Matrix	z-scores
EURL-PC 2022-09	Acrylamide	Cereal-based infant food	-0.7
EURL-PC 2022-10	Furan and alkylated furans	Cereal product	Between 0.3 and 2.0; *12.2 for furan*
EURL-PC 2022-11	Free and bound 2- and 3-MCPD and GE	Powdered infant formula	Between -0.5 and 1.8
EURL-PC 2022-12	Free and bound 2- and 3-MCPD and GE	Baby biscuit	Between -0.5 and 1.5
FAPAS 30119	Furan and alkylated furans	Coffee	Between -0.7 and 0.5
FAPAS 06105	PAHs (benzo[a]anthracene and benzo[a]pyrene)	Smoked black pepper	-0.9 resp. -0.2
FAPAS 06117	PAHs	Smoked fish product	Between 0.5 and 1.2
FAPAS 2668	3-MCPD esters	Soy sauce	-0.1
FAPAS 2670	2- and 3-MCPD esters	Potato crisps	1.2 and 1.6, resp.
FAPAS 2671	2- and 3-MCPD esters and GE	Infant formula	0.6 and 0.4, resp.; *18.6 for GE*
FAPAS 2672	2- and 3-MCPD esters and GE	Vegetable oil	0.7 and -1.1, resp.; *2.3 for GE*
FAPAS 30122	Acrylamide	Coffee	0.2
FAPAS 30124	Acrylamide	Infant food	-0.1
FAPAS 30127	Acrylamide	French fries	-0.2
Test Veritas B2505	Acrylamide	Bread	1.5
Test Veritas RC2506	Acrylamide	Coffee	-0.4

Most of the results listed in the table above were satisfactory (z-scores between -2.0 and 2.0), yet three were above 2.0. The first unsatisfactory result was a z-score of 12.2 for furan in the EURL-PC 2022-10 PT. Cereal product is a matrix that has not been validated; therefore, there is not much experience with it within WFSR. The sample was analysed according to a standard addition method suggested by the EURL. The

sample was analysed twice due to a mistake in the first analysis. The level of furan in the sample seemed higher in the second analysis than in the first one, but the levels of the other furans stayed constant. A new re-analysis of the same material resulted in a new increase in furan levels. Due time constrains due to the PT deadline, the first result of the re-analysis was reported. After the EURL's evaluation of the results, which led to a deviating z-score, new packages of the sample were sent in by the EURL and this new material was analysed, leading to acceptable results. A reason for the unsatisfactory z-score could not be found.

The following unsatisfactory result was a z-score of 18.6 for GE in the FAPAS PT 2671. As stated by FAPAS, the concentration of glycidyl esters in this test material was low. Only 19 laboratories submitted quantitative results, and there was a large subpopulation for which the concentration was below their LOQ. All these factors led to a multi-modal distribution of the results and an associated uncertainty that exceeded the critical value. The assigned values were therefore for information only. No further action was taken on this deviation.

The last unsatisfactory result was a z-score of 2.3 for GE in the FAPAS PT 2672. The raw data and initial calculations were checked, and no errors could be found. A re-analysis of the sample yielded acceptable results. An apparent reason for the unsatisfactory z-score could not be found.

11.2 Assistance to official laboratories

11.2.1 Quality control

The laboratories of the OL and the NRL merged in 2019, and no new OL for processing contaminants has been selected. Before the merger, the two laboratories (OL and NRL) used different analytical techniques to analyse PAHs (HPLC-fluorescence versus GC-HRMS), and both methods are still in use. Quality assurance was established by confirmation of PAH concentrations in several samples analysed with the former OL's method.

11.2.2 Advice

Since the merger of the OL and the NRL laboratories, advice on analytical measurements, quality, and measurement strategies has been provided regularly.

11.3 Scientific and technical support to the competent authority

In 2022, there were several contact moments between the NRL and the ministries of Agriculture, Nature and Food Quality (LNV) and Health, Welfare and Sport (VWS), NVWA and RIVM concerning processing contaminants in food.

11.4 Contacts with other NRLs

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

12 National Reference Laboratory Halogenated persistent organic pollutants (POPs) in feed and food

Coordinator: Kerstin Krätschmer

12.1 Activities within the EURL-NRL network

12.1.1 Participation in EURL-NRL workshops

In 2022, two EURL-NRL workshops were held, an online two-day workshop on 18 and 19 May and a two-day workshop held at the CVUA in Freiburg and online on 29 and 30 November.

The representatives of the DG SANTE of the European Commission gave an update on regulatory issues and developments regarding food and feed at EU level. This included:

- *RASFF notifications*:
  Since November 2021, there have only been six RASFF notifications under the scope of the EURL POPs, one in food and five in feed:
  - Goose breast fillets and thighs (Hungary, PCDD/Fs)
  - Palm fatty acid (Germany, PCDD/Fs exceeding action threshold)
  - Fatty acid distillate (Germany, PCDD/Fs exceeding action threshold)
  - Copper sulphate pentahydrate (Thailand, PCDD/Fs and dl-PCBs)
  - Refined fish oil (China, PCDD/Fs and dl-PCBs)
  - 8 batches of corn oil (Poland, PCDD/Fs exceeding maximum level)

  The corn oil from Poland displayed a congener pattern consistent with burning or combustion of PVC; the Standing Committee asked the competent authority in Poland for further investigations.

- *New regulations and recommendations for PFAS:*
  Following the 2021 EFSA Opinion on four PFAS (PFOS, PFOA, PFNA and PFHxS) in food, the European Commission worked in close exchange with competent authorities and the EURL POPs on drafting and implementing maximum levels (MLs) for PFAS on the basis of available occurrence data. The new MLs for the four PFAS in food entered into force on 01 January 2023.
  Commission Regulation (EU) 2022/2388 of 7 December 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs.

  Furthermore, a recommendation on sampling and analytical methods for PFAS in food was published and has been in force since September 2022.
  Commission Implementing Regulation (EU) 2022/1428 of 24 August 2022 laying down methods of sampling and analysis for the control of perfluoroalkyl substances in certain foodstuffs.

  Recommendations for monitoring in feed, in addition to those for monitoring in food from 2021, are still in preparation, but feed has already been added as a possible additional monitoring aim in the food recommendation.

- *Limited review of maximum levels (ML) for PCDD/Fs and dl-PCBs:*
  The review of WHO TEF values for PCDD/Fs and dl-PCBs is ongoing, with WHO and EFSA collaborating in collecting and handling the database used for the re-evaluation. An expert ad-hoc consultation took place on 17-21 October 2022 in Lisbon, Portugal.
  https://www.who.int/news/item/15-11-2022-who-expert-consultation-on-updating-the-2005-toxic-equivalency-factors-for-dioxin-like-compounds-including-some-polychlorinated-biphenyls.

The limited review of MLs for PCDD/Fs and dl-PCBs resulted in a change of the European contaminants regulation, depicting changes in the following commodities:

- Lowering of the ML for milk, and new ML for several new commodities
- Extension of the existing ML for bovine/sheep meat to goat meat, called "meat and meat products from bovine, ovine and caprine animals"
- Extension of the existing ML for hen's eggs to poultry eggs except for goose eggs
- ML for crustaceans, including crabs is now to be applied to the white meat of appendages and abdomen regardless of species

(Commission Regulation (EU) 2022/2002 of 21 October 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of dioxins and dioxin-like PCBs in certain foodstuffs).

- *Overview of new implementing and delegating acts for National Control Plans:*
  As the original Council Directive 96/23/EC, describing control plans for pharmacologically active substances, some pesticide residues and contaminants, expired on 14 December 2022, three delegating acts and three implementing acts have been published to lay down rules for multi-annual national control plans in these three fields (see Figure 12.1 below). For the Dutch NRL POPs and its associated official laboratories (OLs), the regulations concerning contaminants are of importance.

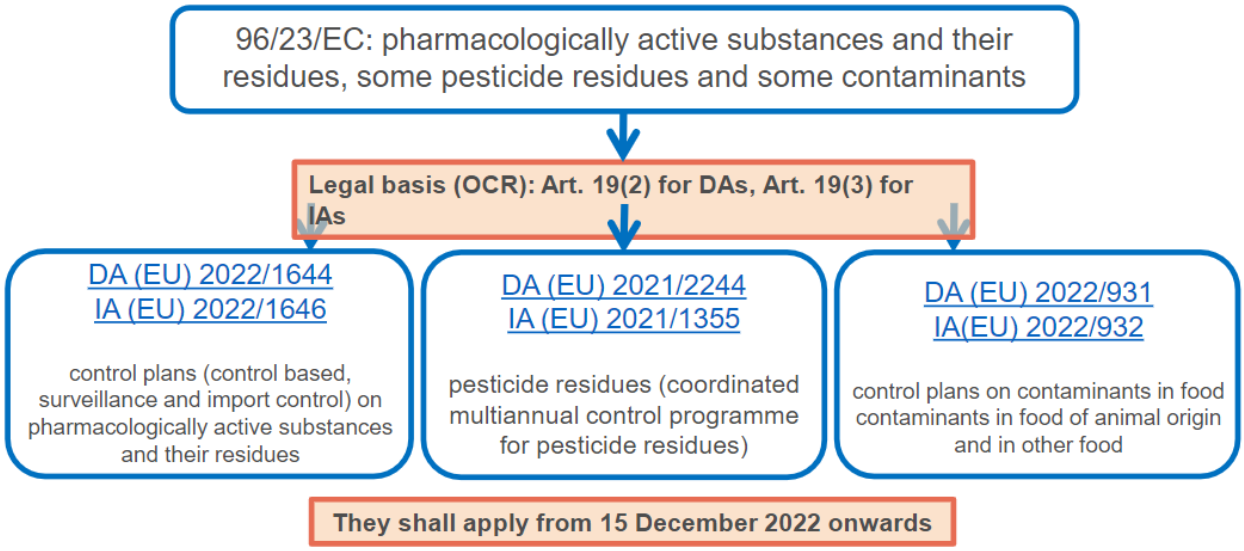

Figure 12.1 *Schematic overview of the Delegating and Implementing Acts (DA and IA, respectively) replacing Council Directive 96/23/EC in the definition of multi-annual control plans.*

Furthermore, the EURL POPs and chairmen and -women of the associated Core Working Groups (CWGs) gave an update on several guidance documents. WFSR, as Dutch NRL POPs, participates in three CWGs (PFAS, Brominated Contaminants and PCNs, and CPs) and chairs two of these CWGs (PFAS, and since June 2022, also CPs). Below is a brief update of their publications.

- *Recommendation for analytical reference standards*
  Joint document of the EURL-NRL network, containing recommendations on the durability of analytical reference standards for analysis of halogenated POPs in feed and food with the focus on expiry dates, handling and quality control. This document is intended for laboratories involved in the official control of POPs. A first draft has been distributed for comments among the EURL-NRL network.

- *Statement on sampling and measurement uncertainty*
  An update was given on the joint statement of EURL-MN, EURL-MP, EURL-PC, EURL-POPs on sampling and measurement uncertainty. In 2022, the European co-operation for Accreditation (EA) contacted DG SANTE regarding the conclusions of this statement pertaining external sampling as part of the laboratory analysis. In a joint meeting, a rewording of this statement was discussed and will be implemented in 2023.

- *EURLs for Contaminants LOD/LOQ Guidance Document*
  The joint document of the former four EURLs in the field of contaminants, first released in 2016, is now available in an updated version with reference to the new additional document, recommending the lowest validated level approach for almost all analytes (provisional exception: chlorinated paraffins). This addendum was published end of 2022, with a joint network meeting of the four EURL/NRL networks in early 2023 to ensure implementation.
  https://eurl-pops.eu/news/guidance-document-loq-2022

- *Guidance Document on the Analysis of Organobromine Contaminants*
  The missing methodology annexes have been added to the guidance document or are in the last stages of preparation: Annex A describes methods for analysis of PBDEs, and Annex B describes approaches for the analysis of HBCDDs. Future considerations for additional annexes include emerging brominated flame retardants (eBFRs) and polychlorinated naphthalenes (PCNs).
  https://eurl-pops.eu/core-working-groups#_bcon-and-pcns

- *Guidance Document on the analysis of PFAS in food*
  An updated version of the guidance document (v1.2) has been published. It now includes an annex providing practical information for laboratories who want to get started with PFAS analysis, or wish to look for alternative approaches. The handling of linear and branched PFOS results has been aligned with EU regulation.
  https://eurl-pops.eu/news/guidance-document-pfas

- *Guidance Document on the Analysis of CPs in food*
  Currently, available performance data, including proficiency tests, ring trials and publications, are being reviewed. Together with the practical work by CWG members, this will form the basis for the missing Annex 4 describing performance criteria for CP analysis, which is expected to be drafted in 2023. In addition, a database of CP homologue group patterns in various food, feed, environmental and human tissue is being developed to identify homologue groups of special importance. The results will be added to the guidance document as soon as they become available.

12.1.2 Participation in proficiency tests and interlaboratory studies

In 2022, the Dutch NRL POPs participated in three interlaboratory exercises (2 PTs and one interlaboratory study) organised by the EURL POPs and two exercises organised by FAPAS. The results are summarised in the following table.

Table 12.1 *PT results of the Dutch NRL POPs in 2022.*

Organiser	Code	Matrix	Analytes	z-scores
EURL POPs	2201-PO	Pork liver	PCDD/Fs, PCBs, (bioassay) PFAS, PBDEs, HBCDDs	-0.1 – 3.0 (0.2) -2.3 – -1.8 not assigned
EURL POPs	2202-FO	Fish oil	PCDD/Fs, PCBs, PFAS, PBDEs, CPs, PBDD/Fs	not assigned (ongoing exercise in 2023)
EURL POPs	2203-FM	Fish meal	PCDD/Fs, PCBs, (bioassay) PFAS, PBDEs	-0.2 – 0.9 (3.9) 0.2 – 5.0 0.0-1.0
FAPAS	6116	Infant formula	PCDD/Fs, PCBs	-0.2 – 2.3
FAPAS	06107	Fish	PFAS	0.0 – 1.3
Quasimeme	DE-18	Seawater	PFAS	-2.9 – 1.7
Quasimeme	BT-9	Fish	PBDEs, HBCDDs	-1.1 – 1.3 not assigned

Of the 230 results reported by the Dutch NRL POPs in the different interlaboratory exercises, 222 were evaluated as satisfactory. The deviating results conformed to the assigned values when the samples were re-analysed after publication of the reports. As PFAS analysis in feed materials has not yet been validated, the

underperformance in fish meal will be taken into account during further method development. HBCDD results could not be reported for the fish oil, fish meal or fish due to a contamination issue in the measurement instrument. The samples will be reanalysed in 2023 for quality assurance. The development of a method with lower (target) LOQs is ongoing. As the fish oil interlaboratory study will continue in 2023, a final evaluation of this exercise is expected by the end of 2023 or early 2024.

12.2 Assistance to official laboratories

12.2.1 Quality control

In 2022, ten specifically prepared milk powder samples were sent twice to the official laboratory (OL) for comparative testing. Results for the ten samples were overall acceptable, although several results in a sample spiked below action level were below LOQ or unsatisfactory due to underestimation, especially in the dioxin fraction. A similar problem was observed in the mono-ortho-PCB fraction of a sample with an unusual congener distribution, where three compounds were underestimated to the extent that they were categorised as below LOQ. While this will not hinder routine compliance testing of the OL, it still hints at an underlying systematic analytical issue. The comparative testing in 2023 will aim to focus more on these issues.

12.2.2 Advice

No particular advice was exchanged with the OL.

12.3 Scientific and technical support to the competent authority

The Ministry of Agriculture, Nature and Food Quality, and the Ministry of Public Health, Welfare and Sport were supported with items mentioned on the agenda of the European Commission, EFSA Opinions, as well as questions about POPs in food and feed.

12.4 Contact with other NRLs

In 2022, the Dutch NRL POPs was in contact with the EURL POPs and other NRLs to share data on PFAS methodology and CP results, significantly contributing to the PFAS guidance document and CP homologue group pattern database. The latter is planned to be published 2023-2024 in a scientific paper.

13 National Reference Laboratory Foodborne 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 (NoV) as a cause of gastroenteritis and hepatitis A virus (HAV) as a cause of hepatitis A, using ISO 15216-1:2017 (quantitative) or ISO 15216-2:2019 (detection). The EURL is based in Uppsala at the Swedish Food Safety Agency. WFSR has been appointed as the NRL for foodborne viruses. WFSR has been analysing the presence of NoV and HAV RNA in several food types, including oysters, under accreditation for over 12 years. RIVM is the NRL for NoV and HAV in bivalve molluscs specifically.

The NRL at WFSR, further referred to as the Dutch NRL, participated in the 5th workshop of the EURL-NRLs for foodborne viruses. The EURL hosted this meeting in Uppsala, 1 -2 June 2022. External guests, among whom a DG Sante representative, were invited to talk about risk assessment calculations for NoV illness due to contaminated food items, especially oysters, and to discuss the next steps in setting microbiological criteria for NoV in oysters.

The EURL presented an update on the draft verification guide for those NRLs that have not yet validated the methods. The EURL verification guide consists of protocols to determine LOD95%, linearity, precision, repeatability and LOQ using artificially contaminated samples with NoV and HAV. Especially NRLs who submitted comments on the previous version will be actively asked to review the next version.

Previous Proficiency Tests (PTs) and future PT schemes were discussed during the meeting. This included the production, quality assurance and obtained results for 21EFV06 (strawberries) and preliminary results for 22EFV07 (swabs from bell peppers). Furthermore, it was decided that PT schemes should cover at least bivalves once a year and seek the possibility of including, besides soft fruit and lettuce, bottled water and food surfaces described in ISO 15216.

In 2020 the Dutch NRL was appointed as project leader for an interlaboratory study to compare NoV analyses using reverse-transcription digital polymerase chain reaction (RT-dPCR) with analyses using reverse-transcription real-time polymerase chain reaction (RT-qPCR), which was carried out in 2021. The Dutch NRL presented the results of the data analyses at this workshop, and the future applicability of RT-dPCR was discussed with the network. The study's outcome will be described in a joint paper of the network led by WFSR and the EURL.

There was a series of presentations on the hepatitis E virus (HEV). One was on the validation of the detection of HEV RNA in Frankfurter sausage (EURL), and another was on the progress of work on the HEV ISO standard. The Dutch NRL presented a recently published monitoring study for HEV in Dutch slaughterhouse pigs. At the end of the workshop, the use of alternative reagents for extraction and detection, still in line with the ISO standard, was discussed, as the SARS-CoV-2 pandemic has shown that reagents can suddenly become hard to obtain.

13.1.2 Participation in working groups

On 31 May, the Dutch NRL participated in the 3rd meeting of the Next Generation Sequencing (NGS) Working Group for the NRLs for foodborne viruses, held as a satellite meeting prior to the NRL workshop 1 -2 June 2022. Presentations were given on different approaches for bioinformatics, molecular NoV surveillance in the past, present and future, and metagenomic approaches to describe NoV diversity in oyster samples. The long-term goal for the Core group of NRLs foodborne viruses with NGS experience, among which the Dutch NRL, is to develop harmonised NGS techniques for typing purposes of foodborne viruses. The Dutch NRL participated in a practical course in NGS on 22-24 November 2022, organised by the France NRL, Ifremer. Protocols for amplicon-based NGS sequencing of NoV in oysters were exchanged. Participation in proficiency and comparative tests

The Dutch NRL participated in six PTs for the detection of genogroup I (NoV GI), genogroup II (NoV GII) and hepatitis A virus (HAV) RNA in food (oysters, strawberry) or lenticule samples. Two were organised by the EURL, two by Public Health England (PHE), one by Bipea and one by FAO/Cefas. Seventeen samples were analysed for three target viruses, resulting in 51/51 (100%) correct detection results. Due to a limited number of participants, not all PT organisers could give scores for the quantitative results. For other PTs, the reports are pending.

Table 13.1 *Overview of proficiency test and comparative studies NRL Foodborne viruses.*

PT (organisation)	Matrix	Number of samples for NoV GI, NoV GII, HAV	Assessment Detection	Assessment Quantification
22EFV08 (EURL, SE)	Bell pepper (swab) 3/22	2	100%	Report pending[1]
22EFV09 (EURL, SE)	Oyster 11/22	3	100%	Report pending[1]
NHV010 (PHE, UK)	Lenticules 3/22	2	100%	100%
NHV011 (PHE, UK)	Lenticules 11/22	2	100%	too few data[2]
83a (Bipea, Fr)	Strawberry 11/22	4	100%	Not done
PT89 (Cefas, UK)	Oyster 7/22	4	100%	75%[3]

1) Report pending, including MAD scores.

2) Not scored as too few participants submitted quantified data.

3) WFSR values were close to FAO ref lab; the median was based on a few participants only.

13.2 Assistance to official laboratories

WFSR is both NRL foodborne viruses and the only Official Laboratory for foodborne viruses in food in the Netherlands.

13.3 Scientific and technical support to the competent authority

During the Annual NRL meeting for Dutch NRLs on foodborne pathogens (i.e. *Salmonella*, *Listeria*, *Staphylococcus*, *E. coli* and Viruses) in October 2022, 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 NRLs

The Dutch NRL organised an interlaboratory study to compare digital RT-PCR with the standardised RT-qPCRs on norovirus detection and quantification in oyster PT samples. Participants were frequently contacted by mail during the study. The study will be reported and submitted for publication in 2023.

14 National Reference Laboratory moisture in poultry meat

Coordinator: Nilüfer Sezer

14.1 Activities within the EURL-NRL network

In 2022, the travel restrictions due to the COVID-19 pandemic again affected the activities of the EURL-NRL expert group 'Water content in poultry meat'. Usually, this NRL participates in two meetings organised every year together with the other European NRLs and the responsible representatives from Brussels. The first meeting is generally scheduled in Brussels, while the second meeting (two-day meeting) is usually hosted by one of the EU members. For the first meeting in Brussels, an online meeting was organised. A second meeting was not scheduled for 2022.

14.1.1 Participation in EURL-NRL workshops

Online expert meeting in Brussels
On March 25, 2022, an online meeting was held of the Expert Group "Water in Poultry Meat". The Dutch NRL presented the results of the 2021 proficiency test they organised. A confidential report was written and sent to the NRL participants. Furthermore, the evaluation and interpretation of national control data from 2020 were discussed, which provided valuable insights. Germany presented the results of their study on the influence of liquid nitrogen on sample homogeneity. After that, an estimation of the physiological water content of chicken breast fillets was presented to the NRLs.

14.1.2 Participation in proficiency and comparative tests

In 2022, the Dutch NRL did not organise or take part in a proficiency test among NRLs. Usually, these tests are arranged every two years after thorough discussions with other NRLs on the scope and design. The next proficiency test is scheduled for 2023, provided that there are enough participants in the study.

14.1.3 Quality control

The Dutch NRL organised one quality control round (February/March 2022) to ensure that the official laboratory (OL) and NRL obtain statistically comparable results for moisture and protein analysis in poultry meat. The quality control comprised an interlaboratory check of the moisture and protein analysis conducted on homogenised chicken fillet meat (n=4) and chicken legs (n=4). The results of the control round showed that one individual sample fell outside the limits of inter-laboratory reproducibility for protein content. The other three values do meet the limits. For moisture, all ground chicken fillet samples met the limits. The average absolute values based on $| WFSR_{value} - OL_{value} |$ for chicken fillet remain within inter-laboratory reproducibility.

Regarding chicken legs, it was found that all moisture contents from WFSR were numerically higher than those from the OL. This was not reflected in the moisture contents of the fillets. The individual differences in protein and moisture content between the labs remained within the limits for chicken legs. The average absolute values based on $| WFSR_{value} - OL_{value} |$ also remained within inter-laboratory reproducibility. This quality control round has shown that, in general, no significant difference between both laboratories has been found for protein and moisture for both fillet and chicken legs.

14.2 Scientific and technical support to the competent authority

The NRL experts provided regular scientific and technical support to the competent authorities, but there were no specific incidents or events that required their consultation.

Wageningen Food Safety Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0)317 48 02 56
wur.eu/food-safety-research

WFSR Report 2023.014

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Wageningen Food Safety Research
P.O. Box 230
6700 AE Wageningen
The Netherlands
T +31 (0) 317 48 02 56
wur.eu/food-safety-research

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